

**LONG TERM CONSEQUENCES OF EARLY
INFANT INJURY AND TRAUMA UPON
SOMATOSENSORY PROCESSING**

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BY

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Abstract

Long Term Consequences of Early Infant Injury and Trauma upon Somatosensory Processing

The aim of the study was to investigate long-term consequences of early infant injury upon somatosensory processing. Quantitative sensory testing (QST) was used as a measure of cutaneous sensory thresholds in areas of scarring and control areas in children that had previously undergone surgery or traumatic intensive care procedures in infancy. The first group tested were adolescents, born premature and cared for in a neonatal intensive care unit (NICU), and the second group were children who had had cardiac surgery in the neonatal period. Results were compared with those from age-matched controls.

The scars resulting from NICU in the first cohort were scarce, on variable locations and small in size. Nevertheless some trends and patterns were seen, but not sufficiently large for a bigger study of NICU survivors with similar degrees of scarring. In this participant group no global difference in thresholds was demonstrated, but in the questionnaire the NICU survivor group had significantly lower scores compared to the control group when reporting amounts of worst pain intensities for common pains.

In the cardiac surgery group participants were less sensitive to touch perception over the reference area compared to the control group and their scar areas were less sensitive for cool, warm and touch perception than any other site tested. They also reported an immediate feeling of “hot” and the missing of “warm” sensation over their scars. Comparing answers of scar and control group in the pain questionnaire, there was no evidence of different pain experience in daily life.

We conclude that tissue injured in early infancy remains measurably altered to mechanical and thermal stimulation in later life and can confirm that early infant injury has not only local, but also global long-term consequences upon sensory processing.

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1. General Introduction

1.1 Painful experiences in infancy – Importance of research into management and consequences

Critically ill paediatric patients are frequently exposed to pain as a result of their disease processes, surgery or intensive care therapies. Historically, the neonate was regarded as incapable of experiencing pain. However, with increased awareness of fetal development and infant behaviour, it is now generally conceded that neonates can experience and respond to pain (Porter and Anand, 1998).

The prevalence and source of pain in paediatric inpatients was examined by Cummings and colleagues (1996) in a tertiary care hospital in Canada. 49% of participants reported clinically significant levels of worst pain and 21% had clinically significant levels of usual pain. Causes of pain were variable and included disease, surgery, and intravenous lines. Children were given significantly less medication than was prescribed, regardless of their reported pain level. Nurses, mothers, and 'no-one' were frequently reported as helping with pain. Medications and non-pharmacological methods were reported as helpful with pain.

Considering the results of this study, which included many children who were able to express that they had pain, one can imagine what would be the outcome of a similar study conducted in a purely non-verbal group, who are not able to communicate their specific needs easily. To complicate things more for the caretaker, behavioural responses of infants to pain stimuli across different developmental ages vary, premature infants are different from older infants, full-term newborns are different from others, but 2-and 4-month-olds are similar (Johnston et al., 1993). For example bodily activity is diminished in preterm neonates in general, relative to full-term newborns

and facial activity increases with the gestational age of the infant (Craig et al., 1993). Cries of the premature infant, however, have more of the characteristics that are arousing to the listener which serve to alert the caregiver of the state of distress from pain (Johnston et al., 1993). In order to detect subtle changes in behavioural state extensive training is required. Behavioural evaluation is extremely problematic in the critically ill or preterm neonate because behaviour may already be altered due to illness or immaturity and behavioural examinations (i.e. Brazelton Neonatal Assessment Scale) cannot be performed on the ventilated neonate. Furthermore failure to discriminate between responses to painful and non painful stimuli is a common weakness of most of the behavioural measures currently available. Large interindividual variability in neonatal responses to painful stimuli suggests that neonates should be used as their own controls to detect changes in response to painful stimuli (Franck and Miaskowski, 1997).

Physiological responses of neonates to painful stimuli include changes in heart rate, respiratory rate, blood pressure, transcutaneous oxygen levels, transcutaneous carbondioxide levels, oxygen saturation, intracranial pressure, and measures of vagal skin tone, skin blood flow, and palmar sweat. Research studies evaluating neonatal responses to painful stimuli have generally included measurement of multiple physiological responses. However, changes in physiological responses are difficult to interpret because these responses are influenced by other non-noxious stimuli (i.e. turning, weighing, bathing), particularly in the ill or premature infant (Franck and Miaskowski, 1997).

Pain assessment is the first step in the provision of appropriate and timely pain management. Over the past decade, numerous pain measures have been developed for preterm and term neonates, however most of them have been developed for

research purposes and have not been tested in the clinical setting (Gibbins et al., 2003).

In recent years there have been major improvements in pain relief for neonates and infants undergoing surgical procedures. De Lima and colleagues (1996) studied the changes in attitude and practice among members of the Association of Paediatric Anaesthetists of Great Britain and Ireland following on from an original survey in 1988. For major surgery in newborn infants (<1 week old), 97 (91%) anaesthetists prescribed systemic opioids in 1995 as compared with six (10%) in 1988. For neonates (< 1moths old) 98 (92%) anaesthetists used opioids in 1995 as compared with 11 (18%) in 1988. Anaesthetists in 1995 who did not prescribe opioids provided analgesia by local anaesthetic wound infiltration or peripheral or regional nerve block followed by paracetamol. Thus in 1995 all infants received analgesics (opioids or local anaesthetic) during major surgery. However, for procedures which are performed outside the operating room, such as at the bedside in NICU, pain is often not managed (Porter and Anand, 1998). Even infants born at term undergo certain unavoidable procedures that are likely to cause discomfort, distress and/or pain. These include vitamin K injections and blood sampling, sometimes repeatedly by heel lancing for diagnostic laboratory tests (McIntosh, 1997). Information published by the Department of Health relating to the years 2002-2003 reports that, out of an estimated 548 000 NHS hospital deliveries in England 8000 (1.5%) were less than 32 weeks gestational age and 32 500 (6%) were between 32 and 36 weeks gestational age (Department of Health Statistical Bulletin NHS Maternity Statistics, England: 2002-03). These small patients are routinely subjected to various diagnostic, surgical or therapeutic procedures that can result in pain (Table 1.1) (Porter and Anand, 1998). The actual incidence of such procedures is not well described: a broad range of frequencies has been reported ranging from as many as three invasive procedures per hour (Pohlman and Beardslee, 1987) to 9 invasive procedures during one week

(Johnston et al, 1997). A longitudinal study of infants studied from admission to discharge in one NICU shows that a total of 144 neonates underwent approximately 7000 procedures, of which in excess of 6000 were heel lancements. On average, only 3% of the total number of procedures was performed with pharmacological pain management specific for the procedure. However, 28% of the total number of procedures was performed with medications that may have helped to minimise the effects of pain. These medications were not given to alleviate the pain of the procedure but rather for other purposes (e.g. to sedate the infant or reduce struggle against the ventilator). Anand and Porter (1998) conclude that despite an increased awareness among clinicians and caregivers regarding pain perception and pain management for infants, and recent advances in the study of neonatal pain mechanisms, the majority of infants still receive inadequate pain management. Many neonates apparently endure unacceptable levels of pain during hospitalisation.

Carlidge and colleagues (1990) studied skin damage resulting from these procedures and found scarring to be present in all 100 NICU survivors at 16-29 months of age. Scarring was usually trivial; however eleven children had cosmetically or functionally significant lesions. The total number of scars was inversely related to gestational age and directly related to the duration of intensive care. Now 14 years later gestational age of the youngest NICU survivor has decreased, procedural possibilities have increased and obviously the overall number of NICU survivors has increased (Dr Judith Meek, personal communication). Therefore we can safely assume tissue damage and scarring have increased as well. Studies have demonstrated that preterm infants in intensive care show prolonged hyperalgesia within an area of local tissue damage (Fitzgerald et al., 1988&1989) and secondary hyperalgesia in the contralateral limb following local ischaemic injury (Andrews & Fitzgerald, 1999).

There has been concern that noxious inputs in infancy, at a critical stage of development during the postnatal period, may have long term consequences for the developing CNS (Anand & Scalzo, 2000); for example developing primary sensory neurons and sensory connections within the spinal cord are sensitive to alterations in normal sensory input (Fitzgerald and Walker, 2002). This has led us to predict that changes in the sensory system occur in human infants exposed to pain, producing altered sensory thresholds and perception later. These changes can be assessed and quantified in long term follow-up studies of patients that have undergone early intensive care or an operation in infancy, using quantitative sensory testing methods (QST).

We aim to study the long term consequences of early infant injury and trauma upon somatosensory processing. Firstly we want to find out if there are any long-term changes and what the extent of these changes might be, no studies in humans addressing these issues have been done up to now. We are further interested if such changes have any influence on people's lives and if they require medical attention. Our research will provide novel information about the plasticity of the human infant somatosensory system. If changes are present, these will provide pointers for further research, for example into the development of therapies to prevent adverse consequences of infant trauma. Eventually, this may lead to a change in medical practice.

The next sections of this introduction will give the evidence and proposed mechanisms for consequences of noxious inputs in infancy, and finally methods for studying long term effects of noxious stimuli in infancy.

1.2 Evidence that early pain experience and injury alters future somatosensory processing

1.2.1 Evidence in humans

The effect on future pain responses following early pain experiences of varying magnitude and number have been examined in numerous studies. In this section they are divided into shorter term changes (days and weeks) and longer term changes (months and years).

1.2.1.1 Shorter term effects

There are some studies which consider the overall experience in NICU and its consequences and other studies which ask questions following more defined insults. The first group seems to show a rather dampened response to pain after a few weeks. A study by Johnston & Stevens (1996) showed that infants who had spent 4 weeks in NICU, and had been subjected to many more invasive procedures, had less behavioural manifestations of pain (heel stick) than the group that had been there for 4 days. Grunau and colleagues (2001) found also a dampened response for behavioural and autonomic pain reactivity at 32 weeks' postconceptional age and the magnitude of this response was again related to the number of previous painful procedures and gestational age at birth. This group also found that early morphine exposure may "normalize" subsequent pain responsiveness.

Oberlander and colleagues (2002), contrary to their expectations from experience with older children with neurologic impairment, did not find any evidence of an altered pain response pattern in infants with proven brain injury in the neonatal period. They compared 2 groups of infants (with and without parenchymal brain injury) 4-6 weeks after birth at a gestational age of 32 weeks and concluded that the injured brain of the

preterm infant has not yet expressed the identifiable differences in pain display and the functional impairment observed at later ages.

Other studies concentrating on somatosensory changes in and around an area of injury in infants found a more increased response to stimulation. Fitzgerald and colleagues (1989) used flexion reflex threshold as a measure of sensation in a group of premature infants born at 27-32 weeks postmenstrual age. The threshold in an area of local tissue damage created by routine heel lances was half the threshold on the intact heel on the other side. This indicated a hypersensitivity to tissue damage analogous to tenderness or hyperalgesia reported in adults. In a double-blind study treatment of the damaged area with the topical anaesthetic cream EMLA was found to reverse this hypersensitivity or in other words increase the flexion reflex threshold. Treatment with placebo had no effect. Their results show that the newborn infant central nervous system is capable of mounting a chronic pain response to local injury, which results in cutaneous sensitization and can be reduced by local anaesthetic.

Andrews and Fitzgerald (2002) studied a group of infants receiving unilateral abdominal surgery and showed a similar enhanced cutaneous sensitivity in the postoperative period around the area of the wound. The reflex threshold at the wound site (elicited by applying calibrated von Frey hairs) was significantly lowered by up to 78% after surgery and subsequently increased to varying degrees based on the type of analgesia used. Thresholds remained below pre-operative values 24 hours after surgery on the operated side. In addition, it was observed that infants in whom the indication for surgery was a chronic condition, displayed lower thresholds on the affected side prior to surgery.

There is also evidence of persistent hypersensitivity following infant surgery. A follow-up study of infants 3 months after corrective surgery for unilateral hydronephrosis

showed that the majority still displayed increased abdominal sensitivity compared to control infants of the same age (Andrews et al., 2002).

Further evidence shows that this sensitisation is not confined to the actual area of injury, but spreads to other regions. Infants, having undergone repeated heel lances in the previous 24-36 hours, showed increased pain reactions (grimace, Visual Analogue Scale (VAS) and crying) to venipuncture on the forearm compared to normal infants (Taddio et al., 2002). This effect extended even to increased grimacing during non-noxious skin cleansing.

1.2.1.2 Longer term effects

Knowledge of the short term effects of early painful experiences is important for immediate clinical pain management however equally important are longer term effects which might have influence on an individual's life for ever. Up to now there are no longer term studies directed specifically towards sensitivity in and around the site of injury itself. A study of infants 3 months after corrective surgery for unilateral hydronephrosis is the longest follow-up done to date addressing these points. It showed that the majority still displayed increased abdominal sensitivity compared to control infants of the same age (Andrews et al., 2002).

There are only a couple more reports addressing altered pain behaviour in general to noxious stimuli later in life following neonatal surgery. A much discussed study of boys circumcised at birth showed that these infants had an increased pain response to routine vaccination at 4-6 months compared to uncircumcised boys. The first group of boys also had significantly longer crying bouts and higher pain scores (Taddio et al., 1995). In a follow-up, prospective study on 87 infants, boys circumcised with placebo cream again showed the greatest pain response at vaccination 4-6 months later,

followed by those circumcised after treatment with lidocaine-prilocaine cream (EMLA), while those uncircumcised showed the lowest responses (Taddio et al., 1997). An examination of behavioural and cardiovascular responses to routine immunization in 14-month-old toddlers, who had gone through major operation(s) shortly after birth, showed that these toddlers responded to the injections with excessively vigorous facial expressions and significantly decreased heart rate (see Lidow, 2002).

Review of studies examining former preterm infants and the effects of numerous procedures in NICU, yields somewhat different results (Johnston et al., 1996, 1999). The decrease in pain responses seen after a few weeks of neonatal intensive care seems to have disappeared after 4 months corrected age. Oberlander and colleagues (2000) compared behavioural and cardiac autonomic response to finger lance blood collection in preterm and term-born infants of this age and the pain responses were similar overall between both groups of infants.

However Grunau and colleagues (1994a) conducted a study of 195 toddlers with varying birth weight from 480 to over 2500g whom they examined at 18 months of age. The children were divided into three groups according to their birth weight and an additional group of full-birth weight children were entered into the study as well. They found that parents perceived the lowest birth weight groups to have the lowest pain responsiveness and that also, unlike in toddlers of greater birth weight, this group displayed no relationship between temperament and pain perception (Grunau et al., 1994a). A further study showed in older children of 4.5 years, that 'somatization', the occurrence of numerous pains that cannot be accounted for medically, was significantly greater in the lowest birth weight children who had experienced lengthy stays in NICU (Grunau et al., 1994b). At age 8-10 years, children of extremely low birth weight (ELBW < or = 1000g), and of full birth weight (FBW) did not differ overall in their perceptions of pain intensity or affect. However the ELBW children rated

medical pain intensity significantly higher than psychosocial pain, unlike the FBW children. Despite altered response to pain in the early years reported by parents of ELBW children, on the whole these children judged pain in pictures similarly to their term peers (Grunau et al., 1998).

Recently in a study of 60 adolescents (12-18 years) born prematurely tenderness thresholds were assessed on 18 tender point sites around the body (points as suggested by the American College of Rheumatology for assessing non-articular tenderness in studies of widespread pain and fibromyalgia) and 4 control sites (forehead, forearm, lateral knee and 3rd metatarsal) and compared with 60 age-matched controls. The prematurely born children had significantly more tender points and lower tenderness thresholds in both, tender sites and control sites, they did not however report any increased pain or stiffness in their daily life. The significance of this finding is hard to assess, but increased tenderness in adults is associated with disabling chronic pain syndromes such as fibromyalgia, which in itself is a complex disease (Buskila et al., 2003).

Ex-premature infants often have more educational, behavioural and emotional difficulties during school age and adolescence as compared to their full-term peers. There is no evidence relating these long-term outcomes to neonatal pain or stress, although such an association may be supported by the studies done by Grunau and colleagues discussed above. Also multiple invasive procedures in premature infants cause marked fluctuations in intracranial pressure leading to early intraventricular haemorrhage or periventricular leukomalacia. In preterm neonates of 24-32 weeks gestation, the incidence of poor neurological outcomes (e.g. severe intraventricular haemorrhage) were reduced by continuous infusions of low-dose morphine, and nursing interventions that provided comfort/analgesia were correlated with improved neurological and cognitive function during latter infancy (Anand and Scalzo, 2000).

In summary there are very little data available of long-term consequences following surgery and only slightly more following NICU, and they are hard to interpret. Anand (2000) proposes that all these studies suggest that painful experiences in late human gestation seem to enhance, whereas painful experiences in early human gestation seem to dampen the behavioural response to subsequent pain. Lidow (2002) states, that the only conclusion that could safely be drawn from the available reports is that the direction of the alterations in responses to pain in neonatally-insulted individuals can vary depending on the circumstances in which the neonatal noxious insult took place and/or the circumstances in which these responses were tested. He also concludes that there is currently no clear answer to the question of whether the long-term alterations in nociception, produced by excessive neonatal noxious stimulation, are permanent or only transient in nature. Grunau (2000) stresses the complexity of such studies and the need for careful design and interpretation.

Fitzgerald and Walker (2003) emphasize that the design of all these longer term studies is confounded by many different factors starting at birth with gestational age (Grunau, 1994a) and length of intensive care stay including therapeutic management and stimuli intensity (Johnston and Stevens, 1996; Grunau et al., 2001; Porter et al., 1999). Further on there is evidence that children who are born preterm may have reduced cortical growth (Ajayi-Obe et al., 2000), reduced cognitive test scores and increased incidence of ADHD and other behaviours (Bhutta et al., 2002). Parenting style (Grunau, 1994b) and learning of how to deal with pain (MacGregor et al., 1997) within the family are other major factors when considering why an older child perceives pain the way it does and makes interpretation harder to interpret as time goes by.

Keeping all the confounding factors in mind, we wanted to look especially at consequences that could be measured in a reliable and reproducible manner and therefore decided to investigate long-term consequences of neonatal injury specifically upon somatosensory processing. The idea was to investigate overall changes in sensory perception as well as specific changes of the injured and therefore scarred area of the body. Furthermore we were interested to see if there were any reports of unusual feelings or reaction around the injured area even when the subject had grown up. We decided to use QST for examination of these areas as it is at present the best quantitative and non-invasive method available. No one has looked this far ahead in this population using a relative simple method like QST.

Plasticity of the nervous system can make detection of changes difficult as it can compensate for the adverse effects of altered or excessive inputs (Fitzgerald and Walker, 2003). An animal model showed that rat pups after neonatal nerve transection had significant changes of the innervation of their hindpaw, but the cutaneous nociceptive reflexes showed an almost normal spatial organization (Holmberg & Schouenborg, 1996). In humans it was found that surgical repair of the birth trauma of brachial plexus avulsion results in restoration of sensory function and the localization of this sensation in the dermatomes of the avulsed spinal roots. Also interesting is that it seems no chronic long term pain follows brachial plexus avulsion at birth compared to the often intense pain seen in adults following such an injury (Anand and Birch, 2002).

1.2.2 Evidence in animals

The advantage of animal studies over human studies, especially studies including children and neonates, is that the investigator has the possibility to control

experimental conditions much better, which obviously helps to identify causative factors, and effects of interventions. Mostly rats are used for pain research in neonates. Compared to the human infant the rat is born quite immature and therefore developmental patterns seen in the postnatal period of the rat probably best approximate the third trimester of human gestation, (Narsinghani and Anand, 2000). Therefore it has been proposed that at approximately P10 (postnatal day 10), the brain of the rat is at the same stage of rapid brain development as the human newborn at term. Considering electroencephalographic activity in the human infant of between 35 and 37 weeks' gestation, this corresponds well to the rat of P10 to P13. Consequently one can use the first week of a rat pups' life to model human preterm development (Fitzgerald et al., 1988). Rats are weaned at P21, assumed to be adolescent at P35, and at P60, a full adult (Dobbing, 1981). One has to keep in mind however, that development of various brain regions within a single species is asynchronous and, therefore it would be too simple to estimate the developmental stage of the brain by studying generalized brain morphologic or functional measures (Johnston et al., 2002). Also human behaviour is much more complex than rodent behaviour, for this reason there are limitations to which results from animal studies can inform clinicians. As a result clinical relevance needs to remain tied to specific questions that can be validly answered by animal studies. The specific questions relate to the effects on the central nervous system (CNS) of peripheral injury of differing types and magnitude, how long the effects last, how widespread the changes are (peripheral, spinal, supraspinal), and what mechanisms can block the change, (Johnston et al., 2002).

Several concepts are used to expose animals to pain, which vary in the intensity and duration of effect. These include acute pain models such as single or repeated needle stick (Anand et al., 1999), experimentally induced inflammation (Ruda et al., 2000), cutaneous tissue injury (Reynolds and Fitzgerald, 1995), and nerve ligation (Reynolds

and Fitzgerald, 1992). Pups have to be removed from their mothers during all the procedures mimicking acute or repeated exposure to pain. This separation might introduce important confounding factors in the analysis of the data (Johnston et al., 2002). Maternal care (and pup grooming more specifically) is well recognized to play a critical role in the physiological and behavioural development of the young rat (Levine et al., 1967).

There are various outcomes used when examining the effects of early exposure to repetitive pain. These include tests of mechanical and pain sensitivity, evaluation of stress-related behaviours, or examination of neural substrates affected by pain in fixed post mortem tissues. Most common used is pain sensitivity in response to thermal, mechanical, or inflammatory pain stimuli. The responses can indicate spinal or supraspinal involvement. Reflexive responses such as paw withdrawal in a thermal stimulation are indicative of spinal cord involvement, whereas more complex responses such as paw-licking, or self-grooming after formalin injection involve supraspinal mechanisms. The implication for clinical practice is important because behavioural differences seen in survivors of NICUs involve almost always supraspinal and CNS-mediated mechanisms (Johnston et al., 2002).

1.2.2.1 Behavioural studies

Finding animal models where tissue injury or repeated noxious stimulation in the newborn pup results in changes in adult sensory behaviour is one of the first steps when studying consequences of early infant pain upon future somatosensory processing (Fitzgerald and Walker, 2003). Such models have been reported and at the same time have shown the complexity of design and interpretation of such studies. It is most important to choose a neonatal pain stimulus of the appropriate

intensity and duration, and to decide when and how to test the effects (Fitzgerald and Walker, 2003).

One of the earliest studies examined the long-term effects of footshocks administered to rat pups' hind paws twice a day from postnatal day zero to postnatal day 21 (P0-P21). This resulted in a significant increase in paw-lick latency in hot-plate tests as well as an enhanced morphine-induced analgesia in the adult rat (P90-100) (Shimada et al., 1990). The main restriction in relating this animal model to human conditions is that it is based on noxious stimuli repeated for an excessively prolonged period after birth (Lidow, 2002). Expressed in human developmental equivalents, this period would be the equivalent to a time span from prenatal week 24-25 to the beginning of the second year of life (Fitzgerald and Anand, 1993).

The long term effects of an injection of inflammatory agent at birth are very much under discussion and seem very much dependant upon the agent and dose used (Fitzgerald and Walker, 2003). A report of Marsh and colleagues (1999) shows a fall in nociceptive thresholds within 3 hours of an inflammatory lesion produced with carageenan in neonatal animals. More interesting though is to find out if the early effects are still present once the peripheral wound has healed. There is one study where the outcome is local mechanical and thermal hyposensitivity in adulthood after neonatal hindpaw injection of 0.25% carageenan in rat pups. However, when the same paws were re-inflamed by an injection of complete Freund adjuvant (CFA), they displayed abnormal hypersensitivity to noxious stimulation (Lidow et al., 2001). The altered pain responses at both baseline and re-inflamed conditions were absent in animals in which the neonatal carageenan injection was accompanied by a 9-hour bupivacaine sciatic nerve block. In contrast other studies using either carageenan or CFA as inflammatory agent did not demonstrate any changes in mechanical or thermal thresholds in adult rats (Ruda et al., 2000; Alvares et al., 2000; Walker et al.,

2003). Alvares and co-workers used 10 μ l 2% carageenan to inject the neonatal hindpaw and produced an inflammation that lasted 2 weeks, but did not find any difference compared with controls regarding mechanical and thermal thresholds at baseline or after re-inflammation. They tested the injured paw and contralateral paw at various ages into adulthood. A later study by Walker and colleagues used 4 different types of inflammatory stimulus (5 or 25 μ l 2% carageenan and CFA each), but also did not find any changes in behavioural thresholds at any stage.

Again using 25 μ l CFA as a strong inflammatory stimulus at birth a further study observes unchanged adult baseline thermal withdrawal latencies but following a repeat inflammation with CFA, hyperalgesia increases very slightly compared with controls (Ruda et al., 2000). It is important to carefully consider the volumes and doses of inflammatory agents used in infant rats for such studies (Fitzgerald and Walker, 2003). For example 25 μ l CFA in a newborn rat leaves a swollen paw into adulthood (Walker et al., 2003) and is not an injury confined to the neonatal period (Fitzgerald and Walker, 2003).

More recently, a different model of repetitive pain was used by Anand and colleagues (1999). This group stimulated rat pups 4 times a day from P0 to P7 with a paw needle prick that resulted in decreased pain latencies when the animals were exposed to a hot plate on P16 and P22, which nevertheless did not persist until adulthood. However, as adults this group of rats showed an increased preference for alcohol and an increased latency in exploratory and defensive withdrawal behaviour. A study by Bhutta and colleagues (2001), however, showed hypoanalgesia in adulthood in the form of increased hot plate and tail flick latency after repeated 10% formalin injections into paws from P1-P7. Preemptive morphine treatment before the first injection of formalin attenuated the effects, but only in males. Furthermore, the situation was

complicated by the fact that rat pups which were given morphine without painful stimuli at birth had greater increases in adult tail flick latencies than rat pups with injury. Neonatal pain (and morphine) decrease alcohol preference in this model, (Bhutta et al., 2001). The reason for these differing results may lie in the stimulus intensities. Formalin is a more damaging stimulus that may lead to some sensory neuron death (Tsujino et al., 2000), whereas repeated needle prick is more likely to result only in local inflammation, (Fitzgerald and Walker, 2003).

One more type of injury that has been used for studies is local tissue damage; this involves cutting a small piece of full thickness skin from a hind paw of the newborn rat pup. This type of wound heals rapidly (Johnston et al., 2002). This model again was found to cause in 2 studies hypersensitivity lasting into adulthood. This was investigated by using von Frey hairs to test mechanical thresholds in the area which had been injured in the neonatal period (Reynolds and Fitzgerald, 1995; DeLima et al., 1999). Wounding only had this effect when it was inflicted in the neonatal period (Reynolds et al., 1995). It was also discovered that this hypersensitivity occurs only one week after the injury and lasted for at least six weeks (DeLima et al., 1999). This group also reports that a local sciatic nerve block with bupivacaine for the first 24 hours after wounding did not affect the onset or magnitude of mechanical hypersensitivity.

One of the latest studies in this field (Ren et al., 2004) used again hind paw inflammation with 0.25% carageenan as a model which produced inflammation for 24 hours. Control animals received either saline or no treatment. Pups were injected at different postnatal ages and testing was done over a prolonged time period. The results showed two distinct patterns of pain behaviour in adulthood. The first finding appeared within the first postnatal week: There was an enhancement of the localised

mechanical and thermal hyperalgesia in the affected paw when re-inflammation was introduced. The second finding did not emerge until the animal was over 4 weeks old: A generalised reduction of baseline mechanical and thermal sensitivity all over the body. Both these findings lasted into adulthood and only happened if the original inflammatory stimulus is applied within the first 10 days of life. The results of this study, which showed both, a localised hyperalgesia and an overall baseline hypoalgesia each with their own time of onset, may help the interpretation of all the previous reports with their different outcomes (Ren et al., 2004).

1.2.2.2 Neurobiological studies

Studies of animal behaviour, whilst important, are influenced by almost as many variables as human studies. It is therefore advantageous to investigate changes at cellular level when looking for consequences of early infant injury upon the development of somatosensory connections (Fitzgerald and Walker, 2003).

In studies of neonatal skin wounding the sensory nerve terminals in the area showed a profound sprouting response, which lasted much longer than the actual injury (at least 12 weeks in the rat) (Reynolds & Fitzgerald, 1995; De Lima et al., 1999; Alvares et al., 2000). Sprouting was confined to A and C nerve fibres, there was no sympathetic involvement. Reynolds and Fitzgerald (1995) also showed that the effect was age dependent as in many of the behavioural studies and was largest in wounds performed at birth and decreased progressively in wounds inflicted at older age. The hyperinnervation of the neonatal wounds involved not only local collaterals from nearby damaged nerve branches, but sprouting of sensory fibres drawn from deep tissue and non-cutaneous nerve-bundles (Alvares et al., 2000). This pain model

seems to be the one with the most long-lasting peripheral and central consequences on neural connections (Fitzgerald and Walker, 2003).

After neonatal hindpaw injection of carrageenan, provoking early peripheral inflammation, a transient influence on the postnatal development of rat primary sensory neuron subtypes was shown, which might influence subsequent central development (Beland and Fitzgerald, 2001). The model used immunostaining of lumbar dorsal root ganglia (DRG) for calcitonin gene-related peptide (CGRP), neurofilament (NF200), and isolecitin B4 (IB4) binding and measured proportions of each subpopulation at P0, P3, P7 and P21 in normal rat pups and in those that had received a unilateral hindpaw carrageenan injection at P1. Following neonatal carrageenan inflammation, the rise in some subpopulations differed from those seen after adult inflammation, although similar levels were found by 3 weeks.

Carrageenan inflammation has also an influence upon the neurophysiological properties of dorsal horn cells, increasing spontaneous activity, causing larger evoked responses, and A fibre sensitization (Torsney and Fitzgerald, 2002). In a further study it was shown that these effects were only acute and were not seen anymore at 3 weeks (Torsney and Fitzgerald, 2003).

In contrast, long lasting changes in spinal neuronal connections can be observed in adulthood after CFA injections in the neonate. These consist of a significant increase in the density of nociceptive unmyelinated and fine myelinated primary afferents on the treated compared to the untreated side in several spinal segments (Ruda et al., 2000). Also fos-like immunoreactivity (Fos-LI) which was used as a measure of neuronal activity in dorsal horn nociceptive pathways was increased, (Tachibana et al., 2001). Walker and Fitzgerald (2003) propose to interpret such central changes by considering the very severe tissue damage that has been caused by large volumes of

inflammatory agents which may have neuropathic and systemic consequences and produce an inflammatory response that lasts into adulthood.

Central changes are also described following a model that uses neonatal colonic irritation with formalin between postnatal days 8 and 21 (Al-Chaer et al., 2000). Examination of the abdominal withdrawal reflexes in response to colorectal distension and electrophysiological activity of spinal viscerosensitive neurons was conducted every 2 weeks, between P35 and P90 in anaesthetized, intact rats. These examinations showed significant chronic visceral hyperalgesia, associated with both, elevated background activity of viscerosensitive neurons at L6-S1 level of the spinal cord and increased responsiveness of these neurons to colorectal distension. Furthermore, the same neurons also displayed increased responsivity to noxious stimulation of their somatic receptive fields located in the perianal region, on the flank and upper thigh, and at the base of the tail. Since the observed hypersensitivity of spinal cord nociceptive neurons to somatic stimulation cannot be explained by previous peripheral somatic injury, this hypersensitivity seems to indicate that the long-term changes in nociceptive systems produced by neonatal colonic irritation include persistent central sensitization (Al-Chaer et al., 2000). Thus the long term effects of neonatal visceral stimulation appear to be even more profound than those following cutaneous stimulation.

1.3 Possible mechanisms that underlie long-term changes

There are many possible sites in the nervous system where long-term somatosensory changes could take place, at receptors, primary sensory neurons, spinal cord neuronal circuits, or in the brain (Figure 1.1). They may also involve the pain/stress-

responsive systems (e.g. along the hypothalamic-adrenal-axis) involving a multitude of transmitters, modulators and hormones. It is proposed that activity dependent changes are responsible for altering somatosensory processing in the developing infant (Fitzgerald and Walker, 2003). There is considerable evidence that alterations in normal activity patterns during development due to excessive sensory input can permanently alter the future pattern of connections within the CNS. One of the well researched areas is the influence of sensory experience in the rodent trigeminal system upon the formation of somatosensory synaptic connections. Receptive field reorganization in the brainstem, thalamus and cortex are the consequence of alterations in whisker stimulation during a specific phase of postnatal development (O'Leary et al., 1994; Fox, 2002; Kaas and Catania, 2002). This time span of plasticity is brief, straight away after eye opening for the visual cortex (Berardi et al., 2000) and directly postnatal for whisker barrel formation (Fox, 1992). These are the periods just after the sensory stimulus is perceived for the first time in life. Normal patterns of connectivity are disturbed when activity is modified during this critical period (Fitzgerald and Walker, 2003). Feldman and colleagues (1999) and Sanes and Yamagata (1999) proposed a mechanism for this phenomenon: They suggest concurrent pre and post-synaptic activity reinforces synaptic connections whereas uncorrelated connections will be weakened and eradicated. The molecular basis of this mechanism may include the induction of NMDA-dependent long-term potentiation and depression (LTP and LTD) (Fox, 2002). In this section the literature examining possible mechanisms will be reviewed by starting at the insult "end" of peripheral tissue injury.

1.3.1 Changes in sensory connections

1.3.1.1 *Peripheral Level*

A study conducted in humans by Hermanson and colleagues in 1987 implied that superficial skin wounds evoke in the first instance extensive sprouting of nerve fibres upon reinnervation and that later in the healed wound the sensibility and distribution is back to that of normal unoperated skin. The neural components seem to be involved in the process of wound healing (Zhang and Laato, 2001).

Innervation after neonatal skin wounds, investigated by Reynolds and Fitzgerald (1995), demonstrated that full-thickness skin wounds in the hairy skin of the foot in neonatal rats resulted in pronounced hyperinnervation of the tissue, involving both myelinated A- and unmyelinated C-fibres. Interestingly the effect was greatest when wounds were performed at postnatal day (P) 0 or 7 and declined when performed at P14 and 21, the latter resembling the transient effect in the adult human. There is the possibility that this fact is connected with the observation by Chong and colleagues (1992) that until the age P10 mRNA for growth-associated protein continues to be expressed in sensory neurons. Persistent hyperinnervation into adulthood long after wound healing is further evidence that the final pattern of innervation is determined over a critical postnatal period (Baccei and Fitzgerald, in press).

Application of bupivacaine during sciatic nerve injury does not prevent sensory nerve sprouting in response to skin loss in the neonate therefore sprouting seems to happen unrelated to neuronal electrical activity or sensory transmission (De Lima et al., 1999). There is instead the possibility that neurotrophic factors released from the damaged area may be involved in this strong sprouting response. Constantinou and colleagues

(1994) observed a significant increase of nerve growth factor (NGF) after skin wounding at birth, which was not observed if wounding was performed at older ages. Hyperinnervation within the wounded area coincides with this upregulation (Reynolds and Fitzgerald, 1995). On the other hand anti-NGF treatment failed to prevent neurite outgrowth towards injured skin in vitro and therefore it is unlikely that an increase of NGF alone is the cause of sprouting (Reynolds et al., 1997). A mechanism not involving NGF as a mediator (Jonakait, 1993) releases immune cytokines, which are proposed to directly affect neurite outgrowth, after tissue injury from macrophages, neurons and glia (Kannan et al., 1996).

Apoptotic mechanisms may be involved in changes of neuronal connections after peripheral injury as cells positive for the apoptotic marker TUNEL appear in dorsal root ganglions within one day after nerve transection at P0 (Oliveira et al., 1997; Whiteside et al., 1998). This theory is supported by the fact that sciatic nerve transection in the neonatal rat causes a rapid cell death of ~75% of axotomized neurons, while the degree of cell death (30%) was much lower in the adult (Schmalbruch, 1987; Himes and Tessler, 1989).

1.3.1.2 Spinal Level

There is a critical postnatal period when the spinal cord dorsal horn experiences a fine-tuning of its somatosensory mapping. This is confirmed by the fact that primary afferent A-fibres extend to laminae I & II of the dorsal horn at early postnatal stages, followed by a gradual withdrawal down to lamina III and below within the first three postnatal weeks (Fitzgerald et al., 1994; Beggs et al., 2002). There is also a progressive reduction in A-fibre input to substantia gelatinosa (Park et al., 1999; Nakatsuka et al., 2000) and a slow but sure decrease in dorsal horn cell cutaneous receptive field size (Fitzgerald and Jennings, 1999; Torsney and Fitzgerald, 2002).

Various studies have been conducted to look at mechanisms that can interfere with this development.

Peripheral nerve injury resulted in cell death in the neonatal dorsal root ganglion which was accompanied by a reduction of central terminals in the dorsal horn of the spinal cord (Fitzgerald and Vrbova, 1985). Interestingly, adjacent intact peripheral inputs showed significant collateral sprouting up to such a degree that the innervation territory of the saphenous nerve extended to twice its normal size following sciatic nerve transection at P1 (Fitzgerald, 1985b; Fitzgerald et al., 1990). However sprouting, involving fibres expressing substance P, was only seen after injury during the first postnatal days, getting progressively weaker and disappearing if injury was inflicted at P10 (Fitzgerald, 1985b; Reynolds and Fitzgerald, 1992). There is evidence to suggest that this is not a purely anatomical change, but that the cutaneous afferents develop functional synaptic connections when they spread into neighbouring denervated regions of the spinal cord. In one study the saphenous nerve was electrically stimulated after sciatic nerve transection at birth. In the normal cord, electrical stimulation of the saphenous nerve evokes dorsal horn spikes in L2 to caudal L4. Few or no spikes are evoked in L5. After neonatal sciatic nerve section, saphenous nerve stimulation evoked spikes throughout segments L2 to L6 (Shortland and Fitzgerald, 1991). It is still uncertain to what extent the function of nociceptive processing is influenced by collateral sprouting. There is the possibility of a lasting change in the nervous system with a disproportionate commitment to inputs coming from around the injured, denervated skin. This could be useful in the short term to compensate for lost sensory input from the injured body area, but the long term effects of a permanent alteration in the sensory mapping of the body may be detrimental (Baccei and Fitzgerald, in press).

More recent work has shown that chronic, local exposure from birth of the dorsal horn of the lumbar spinal cord to an NMDA antagonist prevents the normal functional and structural reorganisation of A fibre connections (Beggs et al., 2002). It was found on electrophysiological examination at eight weeks of age that dorsal horn cells in animals treated by a spinal NMDA antagonist had significantly larger cutaneous mechanoreceptive fields and greater A fibre evoked responses, whereas C fibre evoked responses were unaffected. The same difference was seen anatomically, C fibre afferent terminals and cell density in the dorsal horn were unaffected, but exposure to the NMDA antagonist prevented the normal structural reorganisation of A fibre terminals in the spinal cord, there was no postnatal withdrawal of superficially projecting A fibre primary afferents to deeper laminae. There was also an influence on pain behaviour which showed in spinal NMDA antagonist treated animals significantly reduced reflex thresholds to mechanical stimulation of the hindpaw. The results indicate that the normal postnatal structural and functional development of A fibre sensory connectivity within the spinal cord is an activity dependent process requiring NMDA receptor activation (Beggs et al., 2002).

Fitzgerald and Walker (2003) suggested therefore a mechanism by which synaptic connectivity within the CNS may be changed after local neonatal injury, postulating that this injury alters patterns of C fibre excitation, which in turn modifies excitation of NMDA and other receptor systems.

1.3.1.3. Supraspinal level

In comparison to the peripheral and spinal components of nociceptive processing, much less is known about the supraspinal regions that are essential for pain perception. Descending axons from brainstem projection neurons appear to grow down the spinal cord early in fetal life (Leong, 1983). These axons may not, however, extend

collaterals into the dorsal horn to influence sensory processing for some time (Fitzgerald, 1991). Furthermore they do not begin to become functionally effective at inhibiting inputs to dorsal horn cells until postnatal day 10 in the rat and are not fully functional for 3 postnatal weeks (Fitzgerald & Koltzenburg, 1986). This may in part be due to immaturity of neurotransmitter/receptor interactions as well as delayed maturation of critical interneurons. It has been suggested that the maturation of descending inhibition is dependent upon afferent C fibre activity, because rats treated with capsaicin at birth have reduced inhibitory controls as adults (Cervero & Plenderleith, 1985). The lack of descending inhibition in the neonatal dorsal horn means that there is no endogenous analgesic system to 'dampen' noxious inputs as they enter the CNS and their effects may therefore be more profound than in the adult. It also explains why stimulus produced analgesia from the periaqueductal gray is not effective until P21 in rats (van Praag & Frenk, 1991).

In the cortical areas associated with pain processing, it appears that repetitive inflammatory pain in neonatal rats leads to a significant accentuation of naturally occurring neuronal cell death (see Bhutta and Anand, 2002). Specific regions, particularly areas of the piriform, temporal, and occipital cortex show twice as many neurons dying in 1-day-old and 7-day-old rat pups subjected to inflammatory pain compared with age-matched controls, but this vulnerability is not evident in 14-day-old rat pups (see Bhutta and Anand, 2002). Mechanisms leading to these long-term changes may include neuronal excitotoxicity (mediated via activation of NMDA or other excitatory receptors) or apoptosis (mediated via inflammatory cytokine receptors or mitochondrial injury) (Bhutta and Anand, 2002). NMDA-dependent mechanisms not only mediate the spinal transmission of pain but also the long-term effects of pain such as hyperalgesia, allodynia, windup, and central sensitization involved in the pathogenesis of chronic pain states. Accumulating data suggests that exposure to

neonatal pain promotes an increased susceptibility to chronic pain states mediated by NMDA-dependent neuroplasticity (Bhutta and Anand, 2002).

1.3.2 Changes in the stress-responsive systems

There is definite evidence that pain and stress are interrelated at the time of insult. Additionally infants in a NICU setting encounter a lot of stress (maternal separation, impersonal handling, bright lights, excessive noise etc.) which is not perceived as painful per se (Porter et al., 1999). Both, pain and stress have long-term consequences in themselves, but also consequences that again seem to be interrelated (Lidow, 2002). There is much debate about this interrelation and all its possible mechanisms, the main emphasis being the question of whether neonatal exposure to so-called pain-unrelated stress can produce long-term alterations in pain sensitivity (Lidow, 2002; Fitzgerald, 2004). In this section we will discuss the various opinions.

Sternberg and Ridgeway (2003) investigated the long-term effects of prenatal and postnatal stress on adult pain behaviour and adult behavioural indices of stress reactivity in mice. Nociceptive thresholds were increased by prenatal or by postnatal stress (restraint and handling), but not if both were applied. In the latter case, there was an overall reduced stress responsiveness. They concluded that pre-and postnatal stressors have differing effects on the neural circuitry underlying pain, pain inhibition, and stress behaviour. Another study showed a similar result, with increased nociceptive threshold in adult mice after daily neonatal handling (d'Amore et al., 1995). One possible explanation for these changes is that the environmental stress to which these mice were subjected evoked analgesia, known as stress-induced analgesia. This is analgesia unaccompanied by endorphin secretion and not reversed

by naloxone. Possible suggestions for mediation are monoamines (Anand and Carr, 1989), which are transmitters and modulators in the nervous system.

Systematic experimental studies by a Canadian/American group over many years (Meany et al., 1988; Plotsky et al., 1993; Sapolsky, 1997), suggest that the response to stress of the hypothalamic-pituitary-adrenocortical (HPA) axis in the adult animal is shaped very early in life. Newborn rat pups were subjected to “slight stress” – they were taken out of the nest for short periods. Subjected to stress as adults, they reacted with a corticosterone release which was lower and of shorter duration as compared to that seen in non-handled controls. These animals also exhibited a persistent upregulation of hippocampal corticosterone receptors. This enhanced the sensitivity of the negative feed-back system regulating corticosterone release. In contrast to the pups subjected to “slight stress” pups subjected to “severe stress” (maternal separation for 3 hours) showed an increased sensitivity to stress during adulthood. In addition early illness induced by endotoxin, in amounts similar to what can be expected to be released during the course of an infection, increased the organism’s future vulnerability to stress. This shows that several early life events can influence the fine-tuning of the HPA axis (reviewed in Winberg, 1998). Tissue trauma/injury also activates hormonal and metabolic stress responses. The result of HPA axis stimulation is an increase in cortisol and growth hormone and a decrease in insulin (Weatherstone et al., 2003).

A recent very interesting study of Ren and colleagues (2004) demonstrated in a novel way localized consequences of injury at segmental level, and the influence of injury on overall somatic sensitivity. As described previously, this group found hyperalgesia at a wound site emerging within the first week after injury, but additionally a generalised reduction of sensitivity all over the body, only evident after 4 weeks. Both these phenomena only occur if the original insult was within the first 10 days of life and

suggest that early injury is capable of producing changes in the somatotopically-organized circuitry as well as in global neural and/or humoral modulatory mechanisms of pain and stress responsiveness. How do they interact in the long-term? One possibility is that early pain experience enhances stress responses, which in turn increases stress-induced analgesia. Any local sensitisation that might occur at segmental level would be masked by this and require a strong stimulus such as reinflammation to be observed (Fitzgerald, 2004).

Throughout this chapter various studies have been reviewed that provide evidence for long-term consequences of early infant injury and trauma and investigate their underlying mechanisms. A wide variety of injury and trauma can be inflicted on the newborn in the clinical situation, and experimentally on the laboratory animal. This can range from tiny needle pricks to extensive skin injury as in abdominal or thoracic surgery, but also trauma due to a simple change of environment or month long exposure to many hazardous stressful loud noises, bright lights etc. It is evident that the scale of the injury/disturbance, the therapeutic management, and also the timing has all a very important role in determining the eventual outcome. Additionally when considering long-term follow-up studies, in humans (Grunau, 1994b), but also in the rat model (Sapolsky, 1997) the influence of parenting style must not be neglected.

It is possible to measure behavioural, psychological, or physiological outcomes. Up to now, human studies have mainly looked at general behavioural and psychological long-term consequences. Studies show a variety of sometimes contradictory results, often suffering from a degree of difficulty in interpretation, which is not surprising looking at the possibilities of confounding factors. We therefore were interested in a human study looking at physiological long-term consequences that can be quantitatively and objectively measured and decided to examine cutaneous sensory thresholds in injured areas using quantitative sensory testing.

1.4 Quantitative sensory testing (QST)

1.4.1 General overview

Quantitative sensory testing is an assessment of somatic sensory function with the major advantage, especially for children, of being non-invasive. It is possible to apply precise, measured stimuli to exactly the area of skin in which one is interested; there is equipment available to test a wide range of modalities. The endpoint of measurement is either the subject's verbal or motor response or both. It is possible to measure threshold, tolerance or discrimination for the examined modality, depending on the examination protocol. QST enables quantification of sensory changes, which then in turn allows for statistical analysis, e.g. in research, or if measurements are repeated in the same controlled environment, to monitor clinical changes over time. In this section this method and its possibilities and uses will be discussed.

1.4.1.1 Fibres and sensory modalities for testing

There are three main nerve fibre types in the human, myelinated A and B fibres and unmyelinated C fibres, A and C fibres are involved in sensory perception (Table 1.2, Yarnitsky and Fowler, 1995). Cool sensation is evoked by activity in primary afferent units subserved by A δ myelinated fibres (Adriaensen et al., 1983). The neural apparatus that carries the afferent input triggered by non-noxious temperature elevation, evoking warm sensation, uses unmyelinated C fibres (Konietzny and Hensel, 1975; Hallin et al., 1982). This concept is based on direct stimulus/response data gathered through recording electrophysiologically identified single afferents in animals and humans.

Such data also confirms that unmyelinated C fibres carry the afferent input induced by noxious heat, evoking the sensation of burning heat pain (Torebjork, 1974; Hallin et al., 1982). Afferent channels subserved at the primary sensory level by small calibre A δ myelinated fibres may contribute to heat pain sensation. However this seems to be a small contribution (Verdugo and Ochoa, 1992) as heat pain threshold is not altered significantly by the conduction block of A fibres (Torebjork et al., 1984); also heat pain evoked during QST has a burning quality, as opposed to a sharp pricking quality. The former corresponds to excitation of C-polymodal nociceptors while the latter corresponds to excitation of A δ nociceptors in humans (Ochoa and Torebjork, 1989).

Concerning the nature of the neural apparatus responsible for carrying afferent input induced by noxious low temperature ultimately evoking the sensation of cold pain, both A δ nociceptors and C nociceptors seem to be involved, as indicated by noxious low temperature stimulus-response data gathered from recording physiologically identified single afferents in animals (Georgopoulos, 1976). Comparable studies in man are remarkably uncommon (Adriaensen et al., 1983). The sensation of cold pain evoked by appropriately low temperature stimulation is composed of a blend of cold sensation, contributed by input along cold specific thermoreceptors, plus pain sensation contributed by nociceptors. Interestingly, upon abolition of the A δ cold specific component of 'cold pain', the magnitude of the painful component becomes disinhibited and its quality shifts to a striking burning character, in keeping with its C-nociceptor basis (Verdugo and Ochoa, 1992).

Therefore small myelinated and unmyelinated sensory nerve function is assessed by thermal testing whereas large myelinated A β sensory fibres are evaluated with light touch and vibration testing (Siao and Cros, 2003).

1.4.1.2 Equipment

Light touch

For testing this modality von Frey hairs are used. Originally von Frey introduced a method of measuring pressure perception with horsehair in 1898 (Levin et al., 1978). Today a 'von Frey hair' is a single nylon monofilament of graded diameter (0.08-1 mm), inserted at right angle into a perspex handle (Figure 2.1). Sets of hairs are made according to a logarithmic scale of the weight in grams applied when the hair is pressed downwards on a surface (Andrews, 1993). The examiner applies the monofilament on the skin at a right angle, and pressure on the rod is increased slowly until the monofilament buckles or bends. The examiner then asks the participant whether a sensation of touch is felt. Von Frey hairs are especially useful for mapping areas of impaired sensation for which a vibrating stimulus is unsuitable because it spreads to normally innervated skin (Lindblom, 1981).

Vibration

Vibration can be tested with a tuning fork (conventionally 100-128 Hz) and the time to disappearance of vibration sensation measured. Quantification of vibration sense can be obtained using a vibrometer, a device with a stimulating probe that vibrates at a certain frequency (most commercially available models 100 Hz). There are two main ways of recording the sensory threshold. In some devices, the intensity of vibration is increased or decreased by changing the voltage to the stimulator and threshold is then recorded on a scale in Volts (e.g. *Bio-Thesiometer*). Another device exists in which an accelerometer measures the level of vibration in micrometers, which is then recorded as threshold (e.g. *Vibrometer*, Goldberg & Lindblom, 1979). This avoids changes in vibration amplitude due to different tissue consistency (Yarnitsky and Fowler, 1995; Siao and Cros, 2003).

Thermal

All commercially available thermal stimulators use the Peltier principle (Fruhstorfer et al., 1976), discovered by Peltier in 1834. He discovered that when two metal wires of different materials were joined to form an electrical circuit and a direct electrical current was made to flow through the circuit, one junction became cold and the other junction became hot; when the current direction was reversed, the cold junction became hot while the hot junction became cold. Kenshalo and Bergen (1975) introduced this method for the study of temperature sensitivity in human and subhuman species.

The “Marstock stimulator” uses a thermocouple placed in the thermode to measure the temperature. The participant is instructed to press a button as soon as the respective temperature sensation is perceived. This elicits a temperature measurement and recording (Claus et al., 1990; Yarnitsky and Sprecher, 1994). Another commonly used way to obtain a participant's response is the 'yes-no' method.

Mechanical/Pricking pain

Examples for equipment that can be used to test this modality are weighted pinprick devices or an electronic pinprick stimulator. The devices consist of a set of seven punctate mechanical stimulators with fixed stimulus intensities (flat contact area of 0.2 mm diameter) that exert forces between 8 and 512 mN (Baumgartner et al., 2002). The electronic stimulator is an electronic von Frey system with transducer and amplifier. The skin contact is a flat-tipped computer-driven steel probe, various diameters are available. The electronic von Frey hair is handheld and applied with constant increasing pressure, the digitised output of which can be viewed as a ramp on a computer screen (Somedic Sales AB; Moeller et al., 1998).

Pressure pain

Devices for examining pressure pain are a pressure thresholdmeter or an algometer. Algometers, sometimes referred to as 'dolarimeters', 'algesiometers' or 'pressure threshold meters' are designed to quantify and record levels of tenderness via pressure threshold measurement and pain sensitivity via pain tolerance measurement. An Algometer is essentially a very sensitive force gauge designed to measure forces applied to specific locations on the subject (Brennum et al., 1989).

1.4.1.3 Quantification of sensation

QST is a psychophysical test. It requires the participant to be alert, cooperative and able to follow instructions. QST tests the integrity of the entire sensory axis from receptors to brain. Abnormalities in QST parameters do not localize dysfunction to the central or peripheral nervous system, or any particular location along the peripheral nervous system (Chong and Cros, 2004), however QST has been used to study sensory pain mechanisms and their possible location (LaMotte et al., 1991; Koltzenburg et al., 1992; Morris et al., 1997; Baumgartner et al., 2002). Sensory experiences can be quantified through several parameters: (i) sensory threshold; (ii) discrimination; (iii) tolerance levels. Threshold is the function that can most easily and conveniently be measured (Yarnitsky and Fowler, 1995).

Methods of threshold presentation

Method of Limits

Stimulus intensity is increased linearly or exponentially from a neutral stimulus, until the participant indicates the first onset of sensation (e.g. either verbal or by pressing a button). The test can also be administered by starting with a perceived stimulus, then

decreasing stimulus intensity until the participant indicates that the sensation has disappeared. These sequences are repeated several times and the mean of all recorded values is taken as threshold. This method is used for both non painful and painful sensations (Chong and Cros, 2004).

Thermal Sensory Limen (TSL)

Stimulus temperature alternates between warm and cold sensations, without stopping at adaptation. The limen between those two thresholds is the range of “no thermal sensation”. This Method is sometimes called MarStock, since development was done in the cities of *Marburg* (Heinrich Fruehstorfer) and *Stockholm* (Ulf Lindblom) (Yarnitsky, 1997).

These two methods include in practice reaction time, which can increase threshold values especially for relatively slower conducted sensations. For thermal sensation, for example, reaction time artefact is larger for warm than for cool, as the former afferents are slower conducting and is even more noticeably for more distal body sites (Yarnitsky and Fowler, 1995). Reaction time is also dependent on factors such as concentration, drowsiness, or boredom, all of which are difficult to control. A “learning effect” with repeated testing is also possible (Yarnitsky and Sprecher, 1994).

Method of levels

The method of levels overcomes the disadvantage of including the reaction time by administering stimuli of predetermined intensity, only after the stimulus terminates, the participant is requested to respond. This can be done in several different ways:

- (i) Answering ‘yes’ or ‘no’ as to whether the stimulus was sensed or not. Following a ‘yes’ the stimulus intensity is decreased, and following a ‘no’ the stimulus intensity is increased. Size of change from one stimulus to the next is termed a “step” (Yarnitsky and Fowler, 1995).

- (ii) The participant may be asked to rate the stimulus against a scale (Yarnitsky and Fowler, 1995).
- (iii) A further alternative is the *Forced-Choice Method* whereby the participant is presented with two opportunities to detect the stimulus, the stimulus being presented in one of them. The subject is then “forced” to choose when the stimulus was presented (Dyck et al., 1978).

Despite being reaction time-inclusive, Dyck and colleagues (1990) found the method of limits to be comparable in accuracy and reproducibility with the method of levels, however the former having the advantage of being much quicker.

1.4.1.4 Quality control

In this section factors that may influence QST testing results will be discussed. Both the range of values obtained from a control population and the test-retest reproducibility of measurements determine the usefulness of individual testing.

Testing Procedure and Equipment

Examiner

Bell-Krotoski and colleagues (1995) when comparing results of Semmes-Weinstein monofilament stimuli did not find that multiple examiners influenced the results negatively; however everyone in the study followed exactly the same protocol.

The examiner's expectancy of a measurement site's characteristics (e.g. painful or not) influence the data in terms of the magnitude but not the reliability (Ohrbach et al.,

1998). The order of site measurement (e.g. control versus pain, also appears to exert an influence on the data (Ohrbach et al., 1998).

Environment

The environment of the test laboratory may influence results (Shy et al., 2003). QST is a psychophysical method and therefore for example distraction (noise etc.) can have an impact on test results, especially when using a reaction-time inclusive method.

Meier and colleagues (2001) found that the influence of ambient temperature on their thermal testing results was insignificant (mean ambient room temperature $22.5(\pm 1^{\circ}\text{C})$, but suggest that it is reasonable to assume that testing in a very cold or warm room will have an influence.

Concerning von Frey hairs, temperature and humidity of the environment, but more so the latter, should be taken into account when using these hairs for measurements (Andrews, 1993). In the clinical environment especially, for example in NICU or when testing in several different environments, humidity and temperature can be variable. It is also not possible to avoid disinfection of the hairs between participants. Although Andrews (1993) showed that these factors influence the loading of von Frey hairs, she also showed that hairs recover to their initial calibration when stored at the same environmental conditions. It is therefore important to perform von Frey hair experiments in the same environment during a particular study (Andrews, 1993).

Time of day

Claus and colleagues (1990) did not find any diurnal variations of thermal thresholds using the methods of limits in 30 participants with diabetes mellitus. Testing was carried out in the morning (6-9 a.m.) and in the evening (4.30-9.30 p.m.) They therefore concluded that testing can be carried out during normal working hours

without significant influence of daytime. Strian and co-workers (1989) tested thermal thresholds seven times per day on 2 consecutive days and also found no significant differences.

Equipment

There are differences between systems; therefore values from one system cannot just be transposed to others (Shy et al., 2003). At present there is not enough published data available to compare the reproducibility of different systems (Shy et al., 2003). Therefore it is mandatory when comparing data to always use the same system.

Stimulus characteristics

A baseline temperature for thermal testing between 31°C and 36°C is recommended, as this is the 'neutral zone of adapting temperature', at which any thermal sensation induced by application of the probe rapidly vanishes by adaptation (Verdugo and Ochoa, 1992). A uniform baseline temperature is essential for comparable results. The reason is that the dynamic properties of warm fibers covary positively with the baseline temperature over a wide range of temperatures, reaching a maximal dynamic sensitivity well above 40 °C. Allowing varying temperatures would add an uncomfortable extra predictor (skin temperature) and thus more variance, which may obscure the intrinsic properties of the neural sensors and of the subject's perception. Instead, this variance is better removed by a standard skin temperature, which at best is a temperature in the indifference range, when the task is easiest for the participant (Kenshalo, 1970; Darian-Smith et al., 1979). Expression of thresholds in either ΔT° or actual values is used in the literature (Hilz et al., 1998; Meier et al., 2001). There are no comments in the literature about advantages or disadvantages of either mode of presentation.

The rate of temperature change of the thermode also affects sensory thresholds: rates of 1°C/s and 3 °C/s give higher thresholds than rates below 1°C/s (see Chong and Cros, 2004). A changing temperature is part of the “method of limits” which is a reaction time-inclusive method, so that the faster the temperature increases, the greater the potential overestimation of threshold.

A larger thermode size gives lower thermal thresholds and less standard deviation of the threshold (Dyck et al., 1993; Hilz et al., 1998a). The reason for this finding is the recruitment of a larger number of receptors because of the larger surface area, and therefore spatial summation is greater (Price et al., 1992); this is especially the case for warm sensation (Schmidt and Altner, 1986).

Testing algorithm

The inclusion of reaction time in the algorithm gives a higher sensory threshold compared with algorithms that exclude reaction time (Yarnitsky, 1997). Different algorithms take a variable length of time to apply (Yarnitsky, 1997), which again influences results due an inverse relationship between time taken to apply them and concentration.

Site of tested area

There are proven topographic differences for testing of all modalities (Rolke et al., submitted). Generally lowest thresholds are found over the face, followed by the hand and leg, with feet having highest thresholds. Warm threshold may be absent in the dorsal foot of older subjects, in whom the first sensation felt is heat pain, which could be explained by a low density of warm receptors, especially in the foot and leg of old people (Dyck et al., 1993).

Participant factors

Age

This is one of the most significant factors influencing QST results reported by many investigators. There are increases in thermal thresholds for especially warmth and cold which are variable depending on the site tested. It seems that elderly persons have an insufficient number of thermal receptors for a specific local perception of warm and cold, but enough of them to perceive the sensation as such on a larger area (Stevens and Choo, 1998). Also thresholds for light touch are increased. Especially notable is the marked increase in vibration perception threshold with age, particularly in men over 50 (Yarnitsky and Fowler, 1995; Hilz et al., 1998b). This increase already starts at the age of 35 (Skov et al., 1998). An explanation for this is most likely an age dependent degeneration of the peripheral nervous system which occurs earlier in men than in women (Halonen, 1986).

Gender

In a study by Meh and Denislic (1994), women showed greater sensitivity for small temperature changes and for heat pain and cold pain. Fowler and co-workers (1987) also observed gender differences for thermal thresholds. Another study found that females rated noxious heat stimuli as more intense than males and in addition were able to discriminate better between the heat intensities. They conclude therefore that the sex-related variation in pain perception is probably related to sensory factors rather than differences in attitude or emotional response (Feine et al., 1991).

Baseline skin temperature

Some investigators conclude that warming or cooling of the skin is unnecessary within the range normally encountered in routine evaluation (Hagander et al., 2000), whereas others have found weak influences of skin temperature on cold ($r < 0.3$, the

higher the temperature, the lower the threshold), but not on warm thresholds and recommend to consider slow skin warming to the thermode baseline temperature (Hilz et al., 1998 and Meier et al., 2001).

Height

Bartlett and colleagues (1998) report a statistically significant increase with increased height in vibration thresholds measured in the foot, and suggest height-related corrections for vibration thresholds in the foot. Assessment of vibration thresholds by Skov and colleagues (1998) confirms this finding; they concluded that this may be consistent with a hypothesis of increasing susceptibility to peripheral neuropathy with increasing nerve length.

Laterality

Pressure pain threshold (PPT) asymmetry was assessed on digits in 12 right and 12 left handed participants. Right-handed participants revealed a clear asymmetry by showing an increased sensitivity at the left hand, however there was no difference in left-handed participants. Behavioural tests of cerebral laterality showed no difference between the two groups. Therefore PPT asymmetry is associated with handedness, but not with cerebral laterality (Pauli et al., 1999). Rolke and co-workers (in press) however did not find any differences in results between the right and left side of the body.

Fear/Anxiety

Rhudy and Meagher (2000) obtained pain thresholds for heat pain before and after induction of fear and anxiety. They divided participants into three groups: (i) fear, induced by exposure to three brief electric shocks, (ii) anxiety, elicited by threat of shock, (iii) neutral, no intervention. Fear resulted in decreased pain reactivity, while

anxiety led to increased pain reactivity, which suggested that findings from animal studies extend to humans.

Menstrual cycle/sex hormones

Isselee and colleagues (2001) found significantly lower pressure pain thresholds in the perimenstrual phase compared to other cycle phases. This confirms earlier studies of Kuczmierczyk and Adams (1986) and Amodei and Nelson-Grey (1989). Findings of Giamberardino and co-workers (1997), who examined different tissue types with electrical stimulation (skin, subcutis and muscle), showed highest pain thresholds always in the luteal phase, whereas lowest thresholds occurred in the periovulatory phase for skin, and perimenstrually for muscle and subcutis.

Vasodilatation/Sweating/ Sympathetic Activity

Hilz and colleagues (1992) assessed the influence of these factors on thermotesting by imitating them with (i) application of a vasodilating ointment (ii) drinking of lime blossom tea and indigestion of acetylsalicylic acid (iii) caffeine intake. They determined baseline thresholds for cold and warm perception and heat pain on the first day and then after influence of each exogenous stimulus (tested on 3 different days) at 3 different body sites. Local vasodilatation had no effect, sweating lowered cold thresholds at the thenar eminence and caffeine lowered warm thresholds and raised heat-pain thresholds on the thenar eminence. There was no difference at other sites. The authors conclude that these tested exogenous interferences do not disturb thermal perception markedly, especially when testing-procedure and parameters are standardised.

Reproducibility of QST results is a very important point. Many QST studies have addressed this issue over time (Hilz and co-workers (1998a) for thermal perception and (1998b) for vibration perception, Meier and co-workers (2001) for thermal and

vibration thresholds and Thibault and co-workers (1994) for touch perception). There is however no consensus on how it should be defined (Shy et al., 2003) and a variety of statistical methods have been used in different studies. In general a reproducible result cannot be obtained without standardized approach to testing, users must be familiar with their equipment, the room should be quiet and without distractions, instructions must be standardized and the test should always be done in exactly the same manner, and ideally the same examiner should do follow-up testing (Shy et al., 2003).

Validation of QST would require comparison with a gold standard, which is not an easy task, because QST is not suggested as a diagnostic test for one particular disease entity (Rolke et al., 2005 in press). QST has been validated by the American Diabetes Association (American Diabetes Association, 1992), however some investigators have shown it is possible to feign sensory loss (Freeman et al., 2003). Nevertheless increased intra-participant variability is one means of detecting this phenomenon (Yarnitsky et al., 1994). For the population on which we intend to use it, it has yet to be validated.

1.4.1.5. Uses of QST in general

This section will highlight the various uses of QST, before we then discuss in more detail the two areas related to our study, wounding and children.

Diabetic neuropathy is a common clinical application of QST, since it is estimated that 50% of diabetics suffer from polyneuropathy (Yarnitsky, 1997). QST is used for therapeutic trials and studies on the natural history and progression of diabetic neuropathy (e.g. Chan et al., 1992, Vinik et al., 1995; Dyck et al., 2000). Since the

introduction of the first Marstock thermal stimulator, there was a marked increase in the use of QST in the clinical evaluation of patients with diabetic neuropathy (Chong and Cros, 2004).

Aside from documenting sensory deficits (hypoesthesia), there are many other clinical uses of QST. QST is the best test available for documenting hyperesthesia (Verdugo and Ochoa, 1992). Neuropathy secondary to renal failure, vitamin B12 deficiency, alcohol abuse, chemotherapy exposure, paraneoplastic syndrome, toxin exposure, human immunodeficiency virus infection, leprosy, immune-mediated disorders, hereditary neuropathy and neurogenic impotence may be monitored longitudinally to detect any worsening or response to therapy (Hilz et al., 1995; Roos, 1977; Gentile et al., 1993; Elderson et al., 1989; Aratani et al., 1993; Bouhassira et al., 1999; Brown et al., 1996; Thomaides et al., 1992; Hilz and Axelrod, 2000; Vardi et al., 1996). Sensory nerve function may also be monitored after nerve trauma and repair (Teerijoki-Oksa et al., 2004). QST abnormalities may also be noted with central nervous system disorders such as multiple sclerosis or cerebrovascular lesion (Hansen et al., 1996; Samuelsson et al., 1994). QST has also been used for diagnosis of carpal tunnel syndrome (Goadsby and Burke, 1994).

QST has been used very effectively in adults to investigate pain mechanisms in experimental studies involving capsaicin or other irritants. LaMotte and colleagues (1992) investigated sensory characteristics and underlying mechanisms with the help of von Frey hairs and a Peltier thermode in the area of injury and an area of secondary hyperalgesia after intradermal capsaicin injection. After-effect on C nociceptors of high concentration capsaicin (injected) is desensitization where as a low concentration (topical) causes sensitization. They also conclude that the unchanged responsiveness of C nociceptors in the skin outside the injection area suggest rather a central than a peripheral mechanism for hyperalgesia in this region.

A further study used QST to evaluate dynamic and static components of mechanical hyperalgesia in human hairy skin after capsaicin and mustard oil application. Static mechanical hyperalgesia, signalled by unmyelinated afferents and evaluated with pressure pain thresholds, was found only in the injured area. The dynamic component, however, evoked by a cotton bud stroke and mediated by large myelinated afferents, extended into an area of surrounding undamaged tissue (Koltzenburg et al., 1992).

Sensory threshold changes have also been demonstrated using QST in the area of secondary hyperalgesia surrounding topical application of capsaicin. This study on healthy adult participants was the first to demonstrate that low threshold sensory perception can be altered in the absence of background pain in this experimental situation (Andrews et al., 1999).

A study looking for evidence that sensitivity changes in rheumatoid arthritis (RA) result from alterations within peripheral and central neurons used capsaicin-induced mechanical hyperalgesia, tested with von Frey hairs, for investigation (Morris et al., 1997). Findings were an area of hyperalgesia larger in RA patients than in controls, the size of this area correlating with a composite score of joint tenderness, suggesting that joint symptoms have peripheral as well as central origin.

Baumgartner and colleagues (2002) suggest two distinct mechanisms for neuropathic pain, central sensitization and partial nociceptive deafferentation, after investigating patterns of sensory changes in 30 patients and human surrogate models with the help of calibrated von Frey hairs and punctate mechanical stimulators.

Thermal testing was also found to be valuable in the assessment of neuropathic pain disorders. After analyzing the QST results of 465 adult patients with neuropathic pain, Verdugo and Ochoa (1992) found that thermal specific (warm or cold) hypoaesthesia and thermal hyperalgesia may occur in the absence of hypoaesthesia for tactile sub modalities served by large calibre afferents. They also describe 13 abnormal patterns of thermal hypo-and hyperaesthesias. This shows that almost any variety or combination of dysfunction and normality regarding the different modalities is possible. Lindblom and Verrillo (1979) employed QST methods in the examination of eleven patients with sustained neuralgia, using thermal and non-noxious mechanical stimuli. They concluded that suprathreshold hyperaesthesia may exist in the presence of normal sensory thresholds.

Nurmikko and Bowsher (1990) showed a deficit of sensory functions mediated by both large and small primary afferent fibres after post-herpetic neuralgia. QST was also used in a study by Sinay and colleagues (2003) to suggest that trigeminal neuralgia is not only a paroxysmal single nerve disorder, as QST abnormalities were found in the symptomatic division of the trigeminal nerve and in the other two branches on the same side, but also that other more central structures may be involved.

Several groups have looked into the role of QST as an outcome measure in clinical trials. Attal and colleagues (2004) found that mechanical dynamic and static allodynia and static mechanical hyperalgesia decreased, but not thermal allodynia or hyperalgesia, when investigating the effects of intravenous lidocaine on spontaneous and evoked pain due to peripheral nerve injury in 22 patients. In another trial by the same investigators (2002) Morphine, investigated for treating central pain syndromes, showed some analgesic effects on some pain components in the short term, but only little promise for long-term treatment. Modalities tested were brush-induced allodynia, static mechanical and thermal allodynia and hyperalgesia (Attal et al., 2002). The

usefulness of epidural motor cortex stimulation (MCS) in patients with drug-resistant chronic neurogenic pain was assessed by Drouot and colleagues (2002) using thermal testing. They were interested especially in finding parameters associated with the favourable outcome of this procedure, and found that patients who had unaltered non-nociceptive thermal thresholds within the painful area had good outcomes. A further study by Dirks and colleagues (2002) investigated the effect of gabapentin on acute nociception and experimentally induced cutaneous hyperalgesia in healthy volunteers. Sizes of areas of secondary hyperalgesia to mechanical and thermal stimulation were used as outcome measures. Gabapentin suppressed established, and prevented development of new cutaneous sensitization. Von Frey hair stimulation was used on areas of secondary hyperalgesia introduced by a heat/capsaicin sensitization model and noxious thermal stimulation on nonsensitized skin as outcome measures to study the effect of oral lamotrigine, hydromorphone and intravenous remifentanyl (Petersen et al., 2003). The latter two agents showed significant suppressing effects on all types of stimulation, whereas lamotrigine did not.

1.4.2 Use of QST to assess skin sensitivity following wounding /surgery

A number of experimenters have used QST to assess pain/nerve function in a skin area after wounding or surgery. In adults Wilder-Smith and colleagues (1996) used electrical stimulation to assess skin sensation, pain detection and tolerance thresholds up to 5 days postoperatively. Their aim was to assess changes produced by segmental spinal sensitization and descending supraspinal antinociception and examine the usefulness of pre-emptive Fentanyl. They concluded that Fentanyl administration augmented supraspinal analgesia and decreased spinal sensitisation.

The same method was used by another group as one of the means to evaluate effects of Morphine and Tramadol on somatic sensory function up to four days after abdominal surgery (Wilder-Smith et al., 1999).

Moiniche and co-workers (1997) used pressure algometry up to 8 days after hysterectomy at the wound site and at a distant site to investigate the time course and the spread of wound tenderness and its relationship to subjective pain ratings. They found increased sensitivity around the wound, but not at a distant site and good correlation with pain ratings.

Stubhaug and co-workers (1997) used von Frey hairs to evaluate punctate mechanical hyperalgesia after surgery. They examined whether induction and maintenance of central sensitization after surgery could be prevented by the use of the NMDA-antagonist Ketamine, which they were able to confirm. Also Richmond and co-workers (1993) used von Frey hairs around a postoperative wound site to assess the effects of morphine given as pre-emptive analgesia and suggested that morphine prevents the establishment of central sensitisation. These two groups evaluated patients/wounds for 48 hours and 7 days respectively.

Some studies in adult patients used QST equipment to examine the cutaneous sensation for a range of modalities in burns. Firstly Ward and Tuckett (1991) used a microcomputer-based sensory testing device to evaluate thresholds for two-point discrimination, pinprick, warming, touch and vibration in 16 patients and 42 controls. They found all threshold measures in patients with burns were elevated above those for control subjects.

A later study by Malenfant and co-workers (1998) looking at tactile, thermal and pain sensibility in adult burned patients is the study with the longest time interval after insult

(at least 18 months), most patients (121) and most relevant analysis with respect to our planned study. The authors tested the injured site of the body and the contralateral control site and used tactile, thermal and pain thresholds for their assessment. The study contained several interesting findings. Sensory thresholds were significantly higher in burned patients than in control participants; and thresholds for touch and cold perception showed the greatest increases. They also looked at the influence of burn depth, i.e. amount of skin damage, on the severity of deficits of the various sensory modalities. Significant sensory losses were found not only in burn sites but also in the non-injured areas suggesting changes in the central nervous system.

Pedersen and Kehlet (1998) examined the reproducibility of primary and secondary hyperalgesia in an experimental burn model of human inflammatory pain. Mild burns were produced in 12 volunteers, and temperature and pain perception thresholds as well as hyperalgesia were assessed with contact thermodes and von Frey hairs and pain intensity with a visual analogue scale at several time points. The conclusion was that the model is a sensitive model of acute inflammatory pain suitable for double-blind, placebo-controlled studies of analgesics.

In neonates and infants under 1 year, assessment with von Frey hairs in connection with wounding has only been done by Andrews and co-workers (2002a and b). Von Frey hairs were applied preoperatively, immediately after surgery and in the clinical follow-up 3 months after surgery to quantify wound sensitivity in infants following abdominal surgery using the abdominal skin-reflex. Andrews and Fitzgerald (2000) had also used the same technique to map the area of secondary hypersensitivity around a surgical wound in infants.

1.4.3 QST in children

QST in children and adolescents has been used much less widely than in adults. There are a few reports involving children with diabetes mellitus and rheumatoid arthritis. Further there are studies investigating normative values, methodology and limitations for thermal, vibration and touch sensation.

Heimans and colleagues (1987) investigated 55 insulin-dependent diabetic children aged 6-19 years and 81 healthy controls aged 6-15 years. They examined vibration perception, using a 'Bio-Thesimeter' and methods of limits, and thermal sensation using thermostimulators and a two-alternative forced choice procedure on the feet. They concluded that both investigations can be carried out easily and are unobtrusive, the reason they opted for QST as a method for examining children in the first place. Their total testing time was 15 minutes and they found that some younger children (< 8 years) had problems concentrating for the full length of time and needed encouragement.

Thirty-five neurologically asymptomatic diabetic children aged 8-16 and 35 age-matched controls were assessed by Abad and co-workers (2002). Warmth, cold and heat-induced pain thresholds were measured in the dorsum of the right arm and foot. They used the method of limits for testing, with a baseline temperature of 32°C for warm and cold and 40°C for heat pain and a temperature increase of 0.7°C/s for all 3 modalities. This paper does not comment on any problems encountered during testing. In 43% of the diabetic children at least one abnormal sensory threshold was detected (defined as value above the 95th or below the 5th percentile of control individuals).

Hogeweg and colleagues (1995a and b) used pressure algometry to assess pain thresholds in children with rheumatoid arthritis. In one study they examined the joint capsules of the wrists, elbows, knees, ankles and the paravertebral soft tissue in 57 patients and 69 healthy controls, aged 6-17 years. Each location was examined three times in a sequential order with short intervals of about 30 seconds. They conclude that direct quantifiable methods of pain assessment such as by pressure algometry are applicable in children aged 6-17 years. In a similar study they use the same technique on 16 patients with inflamed knees and 17 with inflamed ankles aged 6-17 years to suggest that nociceptive input from inflamed joints may have established changes in the peripheral as well as central nociceptive processing system.

Hilz and co-workers (1998a and b; 1996) assessed normative values, methodology and limitations for thermal and vibration thresholds in children and adolescents. For thermotesting this group used a Thermotest combined with a Senselab program (Somedic, Stockholm, Sweden). In their first study they focused on the ability of children to cooperate sufficiently with a psychophysical test like QST and at the same time created normative data for 74 children between the ages of 3.2 and 6.9 years. Warm or cold perception thresholds were determined according to the "method of limits". The stimulus baseline temperature was set at 32°C. Limits of stimulation were 5°C and 42°C. At each tested body site, five warm and five cold stimuli were applied at 4-10 sec. intervals. Thresholds were averaged from the five registered peak to baseline differences. Body sites tested were: lateral dorsal foot, distal medial calf, distal volar forearm and thenar eminence. Mainly a thermode with a stimulating surface of 1.5x2.5 cm was used, but on 2 sites additionally a larger one was used to compare results. A temperature change rate of 1°C/s was used at all tested sites, except the thenar eminence, where in addition a 3°C/s rate was used. After an interval of up to 58 days 35 children were retested. The conclusion from this study was that QST is feasible in 3-7 year old children and reproducibility was sufficient in children

aged 4 and older. Also it is suggested that the simple structured test procedure implied by the methods of limits favoured these results and allowed for a quick threshold assessment. All children understood the task to respond to a steadily increasing stimulus as soon as it was perceived. A higher stimulus velocity gave lower thresholds, as did a larger thermode size. However, a larger thermode size is only recommended for body sites where there is full contact of the stimulator surface with the skin, otherwise thresholds may vary in relation to the variable contact of the stimulating surface with the skin. Pretest skin warming was recommended with skin temperatures $< 26^{\circ}\text{C}^{\circ}$.

The same group (Hilz et al., 1998a) looked at 225 healthy boys and girls aged 7 to 17.9 years. Exactly the same methods were used and the same body sites tested. The study also attempted to obtain normative heat pain thresholds, but too many volunteers refused to take part in this particular part of the study. Conclusions regarding reproducibility, thermode size and pretest skin warming matched those of the first study.

Normative values of vibration perception were determined by Hilz's group in 530 individuals, 73 of them aged 3.3-6.9 years and 223 aged 7.3-17.9 years. Thresholds assessed with a tuning fork were compared to vibrometer values established with the method of limits and determined at the left or right hand and foot. Results showed a moderate ($r = -0.608$) correlation between both devices. There was no influence of body weight, height, gender or age in children and juveniles. Their findings show that quantitative vibrometer testing yields reliable results and can be performed in children as young as three years. Testing does not depend on the side examined nor does it require a standardized skin temperature.

A further study by Meier and co-workers (2001) investigated normative values for thermal and vibration sensation and thermal pain detection thresholds in 101 children and adolescents. This group reported similar results to the previous group (Hilz et al., 1998a and b) and used the same algorithms. Additionally, they made comparisons between the method of levels (MLE) and limits (MLI) for cool and warm thresholds and found, as in adults that MLE produces lower thresholds, and MLI is faster. Both methods were reproducible. Thresholds for cold and heat pain were also established by this group using MLI, but they conceded that they had not considered the emotional impact and reward expectations on a child's response to noxious thermal testing. In their experience QST is readily understood by most children, however some young children may require encouragement and reminding during the performance of tests if they appear inattentive.

Thibault and co-workers (1994) describe normative values for touch perception in 43 children aged 6-12½ years, examined with Semmes-Weinstein monofilaments. Subjects were examined on two separate occasions, 2 weeks apart. The method of limits was used for threshold determination with ascending and descending series. Test sites were bilateral and included arm (C5), index finger pad (C6), thigh (L3) and ankle (S1). Test-retest reliability coefficients were excellent overall, best for the index fingerpad with $r=0.99$ and $r=0.87$ respectively and worst for the thigh ($r=0.46$ (right) and 0.69 (left)). The index finger was the most sensitive site. There was no significant effect of gender, age, and laterality on the results, but the effect of site was significant.

In summary, although there are few QST studies in children, there is sufficient evidence that it is a simple, nonpainful and readily understood method for children. It is a reliable psychophysical test of large-and small fibre sensory modalities, however the following should be adhered to: (i) instruments and corresponding methodologies must be used that have been shown to be reproducible, (ii) testing protocols must be

strictly followed, (iii) the participant has to be cooperative and alert and must be able to follow the instructions.

Although it would have been desirable to use more sophisticated methods of somatosensory evaluation such as brain mapping, fMRI, and MEG, the funding for this was not available. In addition, access and availability to these methods is very limited in this locality, because of huge demand. Therefore QST was deemed to be an appropriate first tool of investigation.

1.5 Statement of hypothesis

- (i) Tissue injured in early infancy remains measurably altered to mechanical and thermal stimulation in later life.
- (ii) There will be reports of unusual pain experience and reactions in and around the injured area.

Table 1.1:

Types of Procedures Performed on Neonates in NICU

Caregiving	Diagnostic	Therapeutic	Surgical
Bathing Feeding Weighing	Auditory evoked potential Arterial stick Cardiac catheter Heel lancing Lumbar puncture Nose/throat culture Physical examination PET scan Retinopathy of prematurity eye examination Ultrasound Urine Catheterisation Venepuncture X-ray	Aerosol treatment Blood transfusion Chest tube insertion CPAP-bagging Dressing change Extubation Gavage tube Insertion Injection Intubation i.v. insertion Line removal Mechanical ventilation Postural drainage Suctioning	Bronchoscopy Central line insertion Closure of abdominal wall defect (gastroschisis) Diaphragmatic hernia repair on ECMO ECMO cannulation and decannulation Exploratory laparotomy for NEC Insertion of peritoneal drain for NEC Oesophagoscopy Open lung biopsy PDA ligation Rectal biopsy Vesicostomy

Legend of abbreviations:

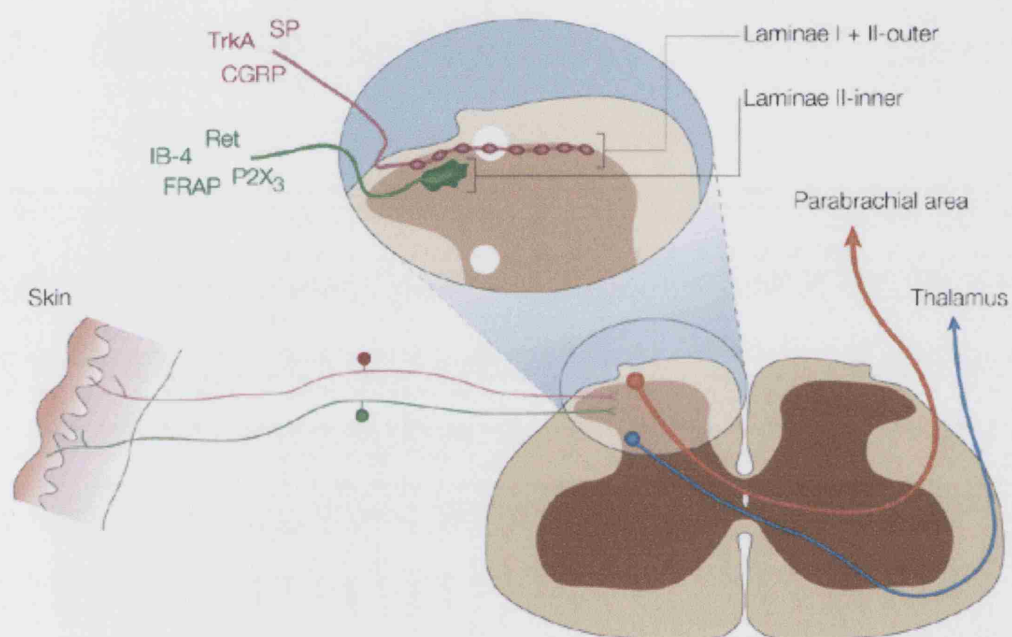
CPAP	Continuous positive airway pressure
ECMO	Extracorporeal membrane oxygenation
NEC	Necrotising Enterocolitis
PET	Positron emission tomography
PDA	Persistent ductus arteriosus
i.v.	intravenous

Table 1.2.: Sensory nerve fibre types in humans

Fibre type	Diameter (µm)	Conduction velocities (m/s)	Sensory modality
A β	7-16	35-70	Mechanoreceptors
A δ	2-6	5-30	Cold receptors Nociceptors (sharp, pricking pain)
C	0.2-2.6	0.4-2.0	Warmth receptors Nociceptors (dull, poorly localized pain)

Figure 1.1: Pain Pathways

a. peripheral and spinal



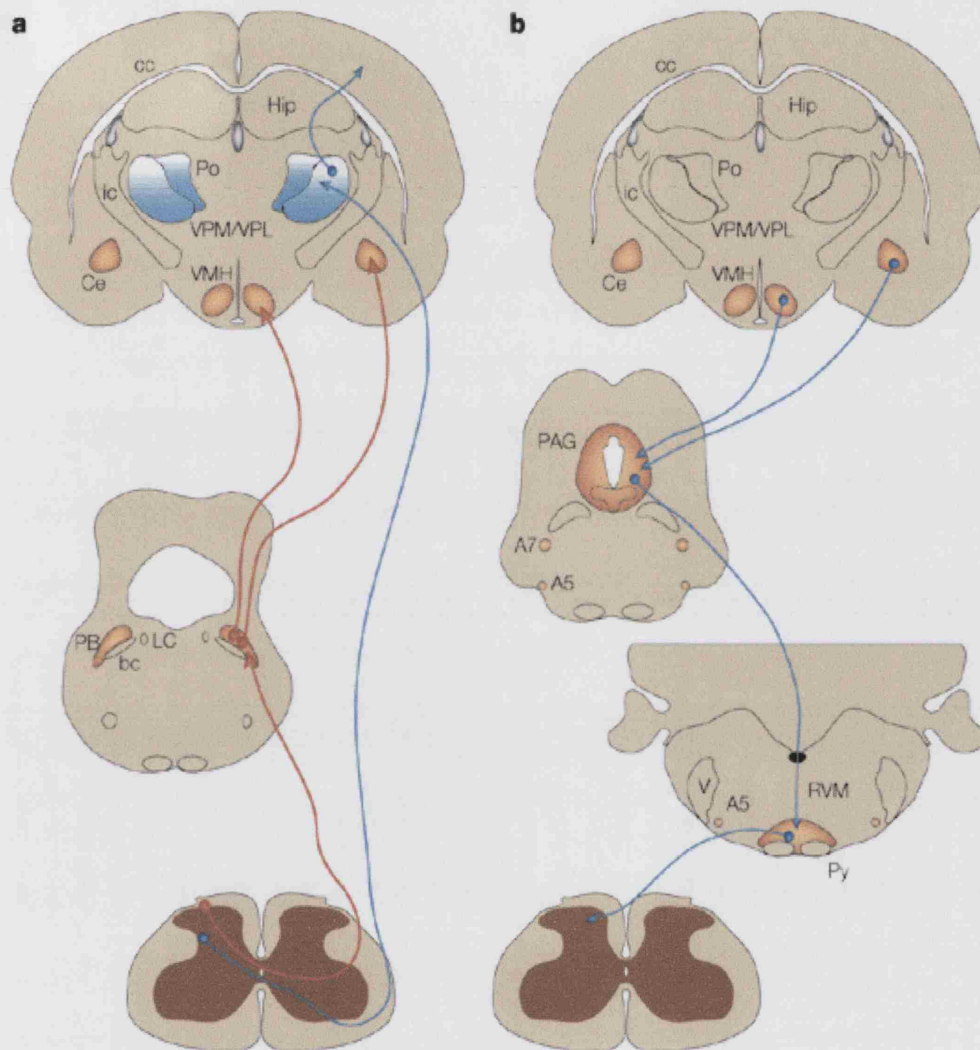
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b. Central Nervous System



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2. Neonatal Intensive Care Unit Survivor Study

2.1 Introduction

In this first study the participant group were adolescents who had been born preterm and as a result needed treatment in a Neonatal Intensive Care Unit (NICU).

This group was chosen as Neonatal Intensive Care survivors have been, for many years, the main group of concern regarding long-term consequences of early pain exposure. Information published by the Department of Health relating to the years 2002-2003 reports that, out of an estimated 548 000 NHS hospital deliveries in England 8000 (1.5%) were less than 32 weeks gestational age and 32 500 (6%) were between 32 and 36 weeks gestational age (Department of Health Statistical Bulletin NHS Maternity Statistics, England: 2002-03). Such preterm infants in intensive care can receive a very large number of invasive procedures and adequate levels of analgesia are frequently hard to gauge (Anand and Porter, 1998). The number of traumatic, invasive procedures in NICU has been monitored by many authors in recent years (Simons et al., 2003; Stevens et al., 2000) and poor levels of pain management extensively reviewed (de Lima et al., 1996; Anand and Porter, 1998). At the time when the participants from our study would have been in the NICU, i.e. 1984-1985, the situation would have been even worse. In their 1988 survey, sent to members of the Association of Paediatric Anaesthetists in the U.K. and Eire, Purcell-Jones and colleagues showed that although most anaesthetists believed that even neonates feel pain, they were reluctant to prescribe analgesia.

Grunau and co-workers (1994a&b, 1998, 2000) have published a series of studies on long term effects of these experiences. In a pioneering study they examined 195 toddlers at 18 months of varying birth weight from 480g to over 2500g, and found that parents perceived the lowest birth weight groups to have the lowest pain responsiveness and that also, unlike toddlers of greater birth weight, there was no relationship between temperament and pain perception (Grunau et al., 1994a). In older children of 4.5 years, 'somatization' (the occurrence of numerous pains that cannot be accounted for medically), was significantly greater in the lowest birth weight children (Grunau et al., 1994b), although this was not observed in a group aged 8-10 years (Grunau et al., 1998). Interestingly, these children also rate pictures of painful events as more painful than their normal birth weight peers (Grunau et al., 1998).

All these longer term studies are hard to interpret due to a large variety of confounding factors such as gestational age at birth (Grunau, 1994a), length of intensive care stay (Johnston and Stevens, 1996), intensity of stimuli (Porter et al., 1999a), therapeutic management (Grunau et al., 2001) and parenting style (Grunau, 1994b). In addition, children who were born preterm can have reduced cortical growth (Ajayi-Obe et al., 2000), reduced cognitive test scores and increased incidence of ADHD and other behavioural disorders (Bhutta et al., 2002). The older the child gets, the harder the studies are to undertake as learned patterns of behaviour within families are a major determinant of perceived sensitivity to pain (MacGregor et al., 1997).

Carlidge and colleagues (1990) showed that iatrogenic skin damage is an inevitable consequence of NICU and was present in each of the 100 children of their study group. The major question is whether such damage resolves with the wound healing or whether, as Anand (2000) claims, experiences of pain will be "remembered" by the developing nervous system, perhaps for the entire life of the individual.

The aim of this project was to investigate any long-term consequences of neonatal injury specifically upon somatosensory processing. Keeping all the confounding factors in mind, we wanted to look especially at consequences that could be measured in a reliable and reproducible manner. The idea was to investigate overall changes in sensory perception as well as specific changes of the injured and therefore scarred area of the body. Furthermore we were interested to see if there were any reports of unusual feelings or reaction around the injured area even when the subject had grown up. We decided to use QST for examination of these areas as it is at present the best quantitative and non-invasive method available.

2.2 Materials and Methods

2.2.1 Participants

NICU survivors were recruited from a cohort of preterm infants who had been inpatients on the NICU at University College London Hospitals. The General Practitioners (GPs) of 25 children born in the year 1985 were traced and contacted (Appendix 2.1). Enquiries were made about the appropriateness of approaching the participants, any chronic diseases they may have, medication they may take and their current address. Following a response from the GP, the NICU survivor's medical notes were examined to determine any factors that might influence somatosensory function. Exclusion criteria were hypoxic/ischaemic cerebral damage as diagnosed by ultrasound scan, neurological abnormalities on previous clinical examination, regular medication or drug use or chronic disease that could influence somatosensory

function (e.g. Diabetes mellitus), and a WISC-R (Wechsler Intelligence Scale for Children) score of <80, indicating cognitive impairment (Roth et al., 2001).

The participants were then sent an invitation letter with brief information about the study (Appendix 2.2). On receipt of a positive response a detailed information leaflet (Appendix 2.3) was sent and an appointment for testing arranged. The participants were asked if possible not to take pain-relieving tablets within 48 hours of the testing appointment, not to take alcohol or other substances of abuse for 12hrs, or smoke for 2 hours prior to testing. Control participants were recruited from friends of the children of the researchers. We aimed to match them regarding age, gender, weight, height and laterality. At the testing appointment, written informed consent was obtained and the participants were made aware of their right to withdraw permission for the continuation of all or any portion of the tests. They were reimbursed their travel expenses and received £20 for participating in the study. All testing was conducted from 14.09.2002 to 07.11.2002.

2.2.2 Procedure

The study protocol was approved by the Joint University College London/ University College London Hospitals Committee on the Ethics of Human Research.

On arrival at the testing session the participants were asked to fill in a questionnaire (Appendix 2.4) giving personal details, marking the presence and location of scars on a body surface diagram, reporting abnormal sensations and pain on and around their scars. We also asked for general pain experience and included a brief medical history, social history and possible use of recreational drugs to make sure we met our in-

/exclusion criteria. Then the scars were inspected and photographs taken of the scars of neonatal origin.

For testing, the participants were seated comfortably in a quiet, draft free room and skin testing sites were exposed. Participant were asked to keep their eyes closed during testing and were not given auditory cues to indicate the start of a stimulus. All participants were given standard instructions and a short demonstration before each type of test to familiarise them with it.

Each NICU survivor was tested on

1. the thenar eminence
2. at up to 2 scar sites
3. at the corresponding sites on the contralateral side of the body as intra-participant control.

Each control participant was tested on

1. the thenar eminence
2. if possible at up to 2 sites where NICU survivors would typically have scars (dorsum of hand and foot, lateral heel) or if they had scars, at these sites.

We limited our testing to these numbers of areas because we felt, when testing pilot participants, that we had reached the end of their concentration span.

We chose the thenar eminence as our reference area, because there are normative values for this area in the literature available which will allow for comparison with our participants (Thibault et al., 1994; Hilz et al., 1998; Meier et al., 2001).

Standardized testing procedures were used for control sites and scar sites. Testing was for mechanical perception and pricking pain thresholds, warm and cool perception thresholds, heat and cold pain thresholds, and brush allodynia.

Participants were informed that they might experience brief painful sensations of pricking, heat and cold and that they could terminate the painful stimulus immediately to avoid undue pain.

Before each series of tests the room temperature was recorded.

2.2.3 Questionnaire

The questionnaire was designed with the aim of gaining information about possible scars as well as overall pain experience using the Adolescent Paediatric Pain Tool (Savendra et al., 1993), the Handbook of Pain Assessment (Turk and Melzack, 2001) and publications by Franck and co-workers (2000). Feed-back on questionnaire design was given by Professor Linda Franck and Dr Amanda Williams.

Before using the questionnaire with the actual participants pilot tests were performed on 5 students from Professor Fitzgerald's laboratory who were of similar age.

2.2.4 Apparatus

2.2.4.1 *Mechanical perception threshold*

Mechanical perception threshold was tested using hand-held von Frey hairs (Bell-Krotoski and Tomancik, 1987) (Figure 2.1). These are nylon filaments inserted into Perspex handles, which are calibrated to apply a certain weight when they are

pressed down on a surface. They are arranged logarithmically in a series of 20 hairs, from lightest to heaviest; the weight in grams and equivalent force in mN for each hair being displayed in Table 2.1.

We decided not to measure vibration as we felt that the scar areas would be too small for this stimulus. The degree of spatial summation with the vibrating stimulus is much larger than with the von Frey Hair (Lindblom, 1981) and therefore the chance of the stimulus spreading to normally innervated skin and therefore influencing the testing results would have been much higher.

2.2.4.2 Thermal thresholds

Thermal thresholds were measured using the Sense lab MSA - Thermal Stimulator (Somedic Sales AB, Sweden) with an 18mm x18mm (3.24cm²) contact thermode (Figure 2.2). The thermal stimulator operates by the Peltier principle. Passing an electrical current through two dissimilar semiconductors displaces heat in the direction of the current. If a heat conductor, such as a metallic plate, is juxtaposed to one side of the semiconductor system, it will be heated or cooled depending on the direction of the current. The plate increases in temperature when the current flows towards it and decreases when the current flows away. The temperature on the surface of the thermode is measured through a built in thermocouple.

The participant received a response button which they were asked to press as soon as a specified temperature sensation was perceived. The digitised output of the thresholds could be viewed on a computer screen (Hewlett Packard HP OmniBook

Notebook PC XE2). The thermode was handheld and the entire stimulating surface was placed in contact with the testing site (Figure 2.2).

2.2.4.3 Mechanical pain threshold

The mechanical pain threshold was identified using a Sense lab Electronic von Frey System Transducer 739 with amplifier 701 and extension box 450-105. The skin contact was a flat-tipped computer-driven steel probe, 0.3 mm in diameter (Electronic von Frey hair, Figure 2.3). The electronic von Frey hair was handheld and applied with constant increasing pressure, the digitised output of which could be viewed as a ramp on a computer screen (Hewlett Packard HP OmniBook Notebook PC XE2).

2.2.4.2 Brush Allodynia

The participants were tested for brush allodynia by applying strokes with a Sense lab – brush with an applied force of 200-300 mN.

Room temperature was measured with a mercury thermometer.

1.5.3 Testing Algorithms

All thresholds were determined on one site first, then moving on to the next site. We tested in all participants the thenar eminence (reference area) first, followed by the scar sites and their contralateral control area. For one scar we started with the scar

site, for the next with the control site to avoid any influence of testing order on the results.

2.2.5.1 Mechanical perception threshold

The following algorithm was used at control sites and at scar sites, both on the scar and at a distance of 3-5 mm from the scar (Figure 2.4):

1. Appearance threshold:

Von Frey hairs were applied sequentially at gradually increasing forces until the participant felt touch.

2. Disappearance threshold:

Von Frey hairs were applied at decreasing intensities starting with the hair noted as “appearance threshold” until no touch was felt.

3. Point one and two were repeated until five appearance and five disappearance values had been obtained.

The filaments were applied perpendicular to the skin for one second until they buckled, maintained in place for one second and then removed. (Bell-Krotoski,1990). The site of stimulation was very slightly altered each time to avoid habituation. The actual threshold was determined as the mean of all ten values.

2.2.5.2 Thermal threshold

The thermode baseline temperature was maintained at 32°C and the cut-off temperature limits were 10°C and 50°C. Cool and warm perception thresholds were determined with decreasing or increasing temperature stimuli respectively, applied at a rate of 1°C/s. The participant was asked to press a button when the specified

sensation was first felt. Pressing the button returned the thermode temperature to baseline temperature at a rate of 2°C/s. First cool and then warm thresholds were determined five times each.

Then four thresholds for hot pain followed by four for cold pain were determined. Again the temperature was increased by 1°C/s, decrease was at 3°C/s. For all thermal thresholds, the mean values of each set of stimuli were taken as the threshold.

2.2.5.3 *Mechanical pain threshold*

Mechanical pain threshold was determined after the thermal threshold as other investigators have found that testing for mechanical pain threshold prior to thermal testing presensitizes the skin and produces false hypersensitivity, especially to heat (Dr Walter Magerl, personal communication). The electronic von Frey hair was manually applied at a ramp rate of 50mN/sec up to a possible cut-off of 1000mN. The participants were asked to press a button when a painful pricking stimulus was first perceived. Then the stimulus was immediately removed. Application of the stimulus was repeated five times with an interstimulus interval of 5 sec. This algorithm was used once at each control site and twice at each scar site, on the scar and at a distance of 3-5 mm from the scar (as von Frey hairs, Figure 2.4). Threshold was taken as the mean of all five values.

2.2.5.4 *Brush Allodynia*

Lastly brush allodynia was tested by stroking each site three times with the brush, using a three second interval between each stroke. The participants were asked to score the sensation with a number between 0-100, with 0 being neutral/comfortable and 100 being most unpleasant/ painful. The mean of three brush scores was taken.

2.2.6 Statistical Analysis

Statistical analysis was conducted using 'MINITAB' software Release 13 for Windows (Minitab Inc., Pennsylvania, PA, USA).

All mechanical perception threshold data were analysed using von Frey hair number units (vFh units; see Table 1), which correspond to a logarithmic transformation (Cole, 2000). These data were analysed in this way because of the heteroscedasticity implicit in using the original von Frey hair scale (Bartlett, 1947).

Group data

Comparison of thresholds for all modalities at the reference area between participants and controls was made using the unpaired t-test.

Data for perception and pricking pain, taking the control area result as 100% and the result on and next to the scar as an equivalent percentage, were analysed with one way analysis of variance (ANOVA, single factor=site), after first checking assumptions

of normality. The results for temperature perception again taken as percentages of the control area were compared with unpaired t-tests.

The frequencies of lowest and highest thresholds at the tested 2 or 3 locations were analysed with Fisher's exact test, using either 2x2 or 3x2 contingency tables respectively.

Individual data

Threshold results from areas on and next to the scars and control areas from the same participant were analysed for perception and pricking pain with one-way ANOVA after first checking assumptions of normality, and Tukey's post-hoc comparisons. The thermal data were analysed using the paired t-test.

Questionnaire

Between NICU survivor group and control group the numbers of reported scars, common pains experienced, the amount of worst pain from these pains reported and the total pain exposure (number of reported scars plus number of pains experienced) were compared with Mann-Whitney tests.

For all statistical tests, $P \leq 0.05$ is considered statistically significant.

2.2.7 Role of MD candidate in the execution of the project

The candidate carried out this project alone, except for some limited assistance from Dr Andrews Campbell. Before the candidate started work on the project funding and ethics committee approval had been obtained. Most of the QST equipment had been purchased. The candidate herself recruited all the participants (without secretarial

help), designed the final QST testing algorithm and the questionnaire. The testing equipment was set up and made operational by the candidate. Testing itself was conducted by the candidate with only 2 participants being tested by Dr Katharine Andrews Campbell. Some pilot testing sessions were carried out together in order to ensure that both experimenters used the same terminology and experimental methods. Some pilot participants were tested by both experimenters blinded and results compared to ensure as little variation as possible between experimenters. The candidate analysed the questionnaire and QST results single handedly and the statistical analysis was undertaken under Dr Andrews' supervision.

2.3 RESULTS

2.3.1 Recruiting of participants

Adolescents rather than children were chosen for the first group so they would be able to consent for themselves and as they are older, be able to concentrate for longer periods of time and give more specific feed-back about the positive and negative aspects of the testing procedure.

The GPs of 25 participants were traced and contacted to check the suitability of approaching the participants and to confirm their current addresses. There were 20 responses (80%) from GPs. After screening the medical notes for exclusion criteria, 13 adolescents were sent invitation letters which resulted in 6 positive responses (46.2%). Altogether 6 participants and 6 controls were examined (n=12).

2.3.2 Demographic of participants

All participants were 17 years old at the time of testing. In the NICU survivor group, the gestational age at birth was in 3 cases 29 weeks, in the other 3 cases 27, 28 and 30 weeks respectively, which resulted in a mean NICU stay of 11.8 days (± 6.62). All participants in the control group were born at term. We were able to match participant factors possibly influencing our results as follows: All participants were of the same age. The NICU survivor group included 5 girls and 1 boy, and the control group 4 girls and 2 boys. 5 of each group were of Caucasian origin, one NICU survivor had parents originally from Sri Lanka and one control participant had parents originally from India. Mean weight of the NICU survivor group was 72 kg (± 12.8) and of the control group 67.5 kg (± 5.4), mean height 165 cm (± 12.3) and 170 cm (± 7.4), all were of average build. The number of right and left handed participants was the same in each group. We did not measure baseline skin temperature, were not able to match females regarding menstrual cycle and did not measure anxiety or fear at the actual testing appointment.

2.3.3 Questionnaire

2.3.3.1 Questions concerning scars

NICU survivors reported between 1 and 7 scars each (mean = 3, $SD \pm 2.19$), one had a scar from an inoculation after the neonatal period, but no other scar. Three had 1-2 scars each at either dorsum of the hand or foot of whose origin they were not sure,

plus 1-2 scars from when they were older. One boy had a definite scar from a radial arterial line in NICU and an older scar, and one girl had 7 scars, 6 of them definitely from her stay in NICU at the back of both hands and feet and both antecubital fossae, plus a further scar from when she was 3 years old.

The control group reported between 1 and 4 scars each (mean = 2, SD±1.26), all received between ages 6-16 years.

No participant in the study reported that a scar was currently painful or caused strange sensations. No neonatal period scar or possible neonatal period scar was reported to ever have been painful or caused strange sensations. From scars received after the age of five, one NICU survivor and one control participant reported one scar which had been painful. There were two older scars in the NICU survivor group and four in the control participant group that had caused strange sensations.

2.3.3.2 Questions concerning pain experience in general

Two of the NICU survivors but no control participants thought that they experienced more pain than their peers, and also had pain that interfered with things they would like to do (headache and migraine). One girl from the control group suffered from cramps in one leg, which interfered with her life. In the rating of how painful their most painful experience was, there was no significant difference between the groups.

The numbers concerning the results of the listed possible pains, their frequency and severity were very small, thus not allowing for direct statistical comparisons.

Comparisons were therefore made between the NICU survivor group (NG) and control group (CG) as follows:

1. The numbers of reported scars per participant

NG: mean = 3, SD± 2.19; CG: mean = 2, SD ±1.26; p = 0.40

2. The amount of worst pain ever experienced

NG: mean = 7.8, SD± 0.83; CG: mean = 7.2, SD ±2.16; p = 1.0

3. Numbers of different pains experienced

NG: mean = 2.33, SD± 1.63; CG: mean = 3.33, SD±2.42; p = 0.51

4. The amount of worst intensity pain from these reported pains

NG: mean = 4.0, SD± 2.35; CG: mean = 7.5, SD±0.83; p = 0.0066

5. The total pain exposure (number of reported scars plus number of pains experienced)

NG: mean = 5.33, SD± 3.44; CG: mean = 5.33, SD±2.66; p = 0.74

The control group experienced significantly greater worst pain intensities with common pains than the NICU survivor group (p = 0.0066) (Figure 2.5).

As a final analysis of general pain experience the results of the amount of worst intensity pain and the total pain exposure of each individual were compared with the results of their thresholds from the reference area. This did not reveal any common trend.

2.3.4 Inspection of scars and scars tested

Of 6 NICU survivors 2 had definite scars from the neonatal period, 3 had possible scars and 1 had none. All scars seemed to result from venous access. The locations of these scars were the dorsum of the hand, the antecubital fossa, the dorsum of the foot and wrist. They were poorly visible and at the most 0.5-1 cm in size. In one girl with definite neonatal scars testing was restricted by the fact that she had scars on both sides and therefore there was no control site available. Due to these factors, 2 definite and three possible scars from the neonatal period were tested.

Other scars tested were located on the volar forearm, chin, forehead, neck, and above the knee. These were all from after the newborn period.

We examined a total of 18 scars, 7 in control participants and 11 in NICU survivors, and where possible the contralateral control sites. In two scars from NICU survivors it was not possible to examine the contralateral site as a control, as one was a midline scar and one girl had a scar on the control site. Furthermore we examined three sites without scarring in 2 control participants at sites where we assumed possible scars in NICU survivors.

The examined scars that were also analysed (n=16) are illustrated in Figure 2.6 and 2.7.

2.3.5 Sensory testing

Overall, the testing procedure was well tolerated. Nobody withdrew from the protocol and nobody was distressed or in severe discomfort. Testing with von Frey hairs for perception threshold was generally said to be most boring and most demanding on concentration, whereas testing for pricking pain with the electronic von Frey was deemed most unpleasant.

When testing scars we were able to test with von Frey hairs and the electronic von Frey both on and next to the scars (Figure 2.4), whereas due to the size of our thermode (18x18mm) this was not possible for any temperature thresholds and the results represent responses to the whole area in and around the scar.

2.3.5.1 Group comparisons of testing results

Thresholds in the reference area (thenar eminence)

Results are shown in Table 2.2. There were no statistically significant differences in the thresholds for mechanical perception ($p=0.463$), cool ($p=0.259$) and warm ($p=0.685$) sensation, hot ($p=0.948$) and cold ($p=0.418$) pain, pricking pain ($p=0.602$) and brush allodynia ($p=0.43$) in the reference area between NICU survivors and the control group.

Comparison of percentage changes of thresholds between older scars and neonatal/possible neonatal scars

For this comparison, threshold results from the contralateral control area were taken as 100%. The threshold results from the actual scar area or area next to the scar were taken as percentages thereof. The results were transformed into percentages as the scars are all on various different body sites and therefore the actual values cannot be compared because of the variability in thresholds at different body sites (Rolke et al., 2005, in press), but it is possible to compare the percentage changes between each scar and its contralateral control area.

The scars were then grouped into neonatal scars/possible neonatal scars (n=5) and older scars (n=11). The mean percentage of each group was calculated and results for each modality compared. This is shown in Figures 2.8-10. There were no statistically significant differences between both groups regarding touch perception and pricking pain thresholds. For thermal thresholds results were as follows: cool: $p=0.830$, warm: $p=0.024$, hot pain: $p=0.886$, cold pain: $p=0.723$. This shows a significantly lower threshold for warm perception in the NICU survivor group compared with the control group.

Comparison of threshold frequencies

Von Frey perception thresholds and pricking pain thresholds

For these two modalities the frequencies of lowest and highest thresholds on the scar, next to the scar and on the contralateral control sites of neonatal scars (n=5) and

older scars (n=11) were compared. The results are illustrated in Figures 2.11 and 2.12.

Concerning von Frey perception thresholds in older scars the frequency of lowest thresholds was highest next to the scar, and of highest thresholds, on the scar. In neonatal scars, frequencies of lowest thresholds were highest on and next to the scar, and of highest thresholds on the scar and on the contralateral control sites. However there was no statistically significant difference between any of these frequencies.

Regarding pricking pain thresholds in older scars, lowest thresholds were most often seen on the scar, and highest next to the scar. In neonatal scars, lowest thresholds were most often seen on and next to the scar, and highest on the contralateral control sites. Again there was no statistically significant difference between any of these frequencies.

Thermal thresholds

For these modalities the frequencies of lower, equal and higher thresholds at scar sites compared with contralateral sites of older (n=11) and neonatal (n=5) scars were analysed. The results are illustrated in Figure 2.13.

For cool perception, the frequency of thresholds higher at scar site is highest in older scars, whereas for neonatal scars the frequency of thresholds lower at scar site is greatest. Again there was no statistically significant difference tables using either between these frequencies for cool perception ($p=0.40$).

For warm perception for both older and neonatal scars, the majority of scars had a lower threshold at the scar site compared to the contralateral site. Fisher's exact test using a 3x2 contingency table was significant at $p=0.034$.

For cold pain threshold for both older and neonatal scars, the majority of scars had a higher threshold at the scar site compared to the contralateral site. However, there was no statistically significant difference between these frequencies for cold pain threshold ($p=1.00$).

For hot pain threshold for both older and neonatal scars, the majority of scars had a lower threshold at the scar site compared to the contralateral site. Nevertheless, there was no statistically significant difference between the frequencies for hot pain threshold ($p=1.00$).

2.3.5.2 Analysis of testing results of individual scars

The results of each of the 16 scars/control areas are shown in Figures 2.14a -2.20p. 9 of these scars belonged to NICU survivors (NSs) (2 definite and 3 possible neonatal scars), and 7 of them to control participants (CPs). In 3 NICU survivors and 1 control participant, 2 scars per participant were tested and analysed. We were analysing all these scars individually to show the variety of perception changes possible. Scars and control areas were also compared inter-participant as there are no normative values in the literature for these particular areas using the same apparatus and approaches.

When looking at touch perception and pricking pain thresholds we compared 48 sites (16 scars x 3 tested sites), for temperature thresholds 32 sites (16 scars x 2 tested sites).

von Frey perception thresholds

In 5 out of 7 tested scars in CPs, the threshold was lowest next to the scar. Amongst NSs this was the case in 2 out of 9 scars, one of them a definite neonatal scar and one of them a possible neonatal scar. In one NS the thresholds next to the scar and on the control area were equal. In 4 NSs the threshold was lowest on the scar (2 possible neonatal scars) and this was also the case in 1 CP. In 2 NSs and 1 CP the threshold was lowest on the contralateral control side. In 8 cases, 4 CPs and 4 NSs (1 possible, 1 definite neonatal scar) the highest threshold was on the scar, in 6 cases (2 CPs, 4 NSs) on the contralateral control site.

In summary on the area next to the scar the threshold for mechanical perception is in 14 out of 16 cases (87.5%) lower than in the other two areas tested. In 8 out of 16 cases (50%) the threshold for perception is highest on the scar (Figure 2.14).

For 27/48 site comparisons (56.3%) of perception threshold there were significant differences in the thresholds between the scar area, area next to the scar and control area. Between scar area and area next to the scar, this ratio was 10/16 (62.5%) (threshold was 8 times higher on the scar area), between scar area and control area 12/16 (75%) (threshold was 7 times higher on the scar area), and between area next to the scar and control area 5/16 (31.25%) (threshold was 3 times higher on the control area).

Cool and warm perception thresholds

Intra-participant differences in threshold are almost equally distributed between those that show no difference between scar and control area (5 scars), those that are higher on the scar (6 scars) and those that are higher on the control side (5 scars). Regarding the two definite scars from the neonatal period, in one case the threshold was higher on the scar, in the other case on the control side.

Concerning warm perception, on 5 occasions, the threshold was higher on the scar, and on 9 occasions, it was higher on the control side (CPs 4/3, NSs 1/6). In 2 NSs it was equal. Regarding the two definite scars from the neonatal period, in both cases the threshold was higher on the control side.

In 5/16 (31.25%) of each, cool and warm perception, there were significant intra-participant differences in thresholds between the scar area and control area.

Hot and cold pain threshold

The threshold for hot pain was in 8 scars higher on the control side and in 6 scars on the scar side (CPs 3/3, NSs 5/3), 2 times it was equal. Regarding the two definite scars from the neonatal period, in one NS the threshold was higher on the scar, in the other NS on the control side.

For cold pain threshold, in 10 out of 16 scars (62.5%) the threshold was higher on the scar side, in 4 scars on the control side (CPs 5/2, NSs 5/2) and in 2 scars the same.

Regarding the two definite scars from the neonatal period, in one case the threshold was higher on the scar, in the other case the thresholds were equal.

In 6/16 (37.5%) cases of hot pain and 3/16 (18.75%) of cold pain there were significant differences in the thresholds between the scar area and control area ($p < 0.05$).

Pricking pain threshold

In 8/48 site comparisons threshold was lowest on the scar, and in 4 scars it was highest. In the area next to the scar, the threshold was lowest in 5 instances and highest in 6 instances. For the contralateral control area, this ratio was 3/6. In 9/48 site comparisons (18.7%) of pricking pain, there were significant intra-participant differences in the thresholds between the scar area, area next to the scar and control area ($p < 0.05$). However, these differences did not seem to follow any obvious pattern with regard to the area tested.

Brush allodynia

One CP felt very slightly uncomfortable when stroked with the brush on one of her scars, she gave this a mean rating of 1.67 out of 100 (from 3 strokes).

2.4 DISCUSSION

2.4.1 Study purpose and design

The aim of this study was to investigate long-term consequences of neonatal injury upon somatosensory processing. In contrast to other studies (Grunau et al., 1994, 1998, 2000), which examined factors regarding pain experiences in general and behavioural aspects, we wanted to focus upon consequences that could be measured in an objective, reliable and repeatable manner. The idea was to investigate overall changes in sensory perception as well as specific changes of the injured and therefore scarred area of the body. Furthermore we were interested to see if there were any reports of unusual feelings or reaction around the injured area.

For our study we used two groups of adolescents all aged 17, one group being born premature and having undergone treatment in NICU, another group being born at term and without any major treatment at hospital in their lives so far.

We started our experiment with a questionnaire in which we asked general questions about health, demographics and substance use to make sure we met our in-/exclusion criteria and that the two groups were matched for gender, ethnic and social background. In addition, we asked all the participants to specify all scars they have, and we asked questions about their scars with the aim to find out about their origin. Finally, we asked the participants questions about general pain experiences.

Then we used quantitative sensory testing (QST) as a measure of perception, measuring thresholds for touch perception with von Frey hairs, for pricking pain with the electronic von Frey hair, and for thermal thresholds with a thermotest.

To compare overall sensitivity changes and to have a reference area, we tested in each participant the thenar eminence. This area has been used in the literature before (Hilz et al., 1998; Meier et al., 2001) and there are reference intervals for thermal testing available.

Our further aim had then been to test in the group of NICU survivors their scars of neonatal origin and as intra-participant control areas, the contralateral site in the same participant. We then had planned to test in the age and gender matched control group bilaterally the same body areas where the neonatal group had had their scars from NICU.

We expected that the NICU survivors would have clearly visible, obvious scars (personal communication Consultant Neonatologist, University College London Hospitals NICU), but unfortunately this was not the case in the majority of our participants. We found that only 2 participants had definite scars originating from the NICU and they were very small. Two more participants had scars of similar size but were unsure of their origin, but due to their location we thought them to be possibly of neonatal origin. All these scars were in varied locations and not easy to distinguish. Due to the lack of scars of neonatal origin but the existence of scars from injuries inflicted at older ages, we decided to test some of these as well. We then went on to test older scars not only in the NICU survivor group, but also in our control group, as we did not have enough neonatal scars in one body area in our NICU survivor group to make direct comparisons of scarred and unscarred areas between groups. Our aim therefore changed slightly to (i) compare baseline thresholds between our two groups as planned originally, but then (ii) to compare in general neonatal scars and older scars and accompanying sensory changes using QST. There are no previous studies of this kind except for one following burns injuries (Malenfant et al., 1998), where a variety of quantitative sensory tests was applied to scars.

2.4.2 Study findings

Despite the fact that we had to modify our original questions we made a number of interesting observations:

1. There was no difference in threshold for any of the modalities we tested at the site of the reference area (thenar eminence) between NICU survivor group and control group.
2. We found a significantly lower threshold for warm perception expressed in % change of control area in the group of scars that were of neonatal, or possible neonatal origin compared to the older scars.
3. When comparing frequencies of lower, equal and higher thresholds at scar sites compared with contralateral sites, in the case of warm perception for both older and neonatal scars, most scars had a lower threshold at the scar site compared to the contralateral site.
4. We found that there are definite changes in perception on both neonatal and older scars and on the immediate surrounding area. We can also state that these changes can last for a minimum of 17 years (the age of our participants). There are certain trends, but no obvious significant patterns of change.
5. In the questionnaire the control group had significantly higher scores compared to the NICU survivor group when reporting amounts of worst pain intensities for common pains.

2.4.3 Interpretation of results

2.4.3.1 *Sensory testing*

There was no statistically significant difference of thresholds in the reference area for any modality between the NICU survivor group and control participant group. Von Frey perception thresholds correlate in both groups with values described as normal perception by Bell-Krotoski and colleagues (1995). The thresholds for cool and warm sensation as well as for hot and cold pain of both groups are within the normal reference intervals for quantitative sensory thermal testing using the method of limits (Hilz et al., 1998; Meier et al., 2001). This result shows that as there is no overall change in baseline sensory processing in our group of participants, experiences in NICU did not result in any basic change in sensory transduction and transmission. Although our sample size was small, if there had been a major effect of early trauma, one might have expected to see it.

We discovered when testing for warm perception a significantly lower threshold when expressed as a % change of control area in the group of neonatal scars. When looking at frequencies of lower, equal and higher thresholds at scar sites compared with contralateral sites concerning warm perception, scars of every age had most lower thresholds at the scar site. These findings were significant when testing, but no participant reported in the questionnaire about a current change of sensation or strange feeling over any of their scars even though we specifically asked for changes regarding different temperature exposure of the skin. This means that the changes we detected with our equipment are not enough to be noticed in daily life. We also did not notice any significant differences for any of the other modalities tested.

The differences we found were in relation to the scar site and not global ones. As changes appeared to be restricted to segmental level, and we did not see any change in baseline sensitivity, we suggest that the mechanisms for this change are to be found directly at the site of injury/scar or in the segmental spinal cord circuitry. Warm perception and heat pain are mediated by unmyelinated C fibres, whereas A α and β fibres convey light touch perception, A δ non-nociceptive fibres cool perception and cold pain is partially mediated by C and A δ fibres (Vinik et al., 1995, Verdugo et Ochoa, 1992). It appears that the cutaneous receptors of these fibres or the circuitry itself seem to be affected by injury and insult in a variable manner as we observed significant differences in comparisons for warm, but not the other modalities. It is well known that inflammatory lesions and skin wounds can cause hyperinnervation and sprouting of sensory nerve fibres into the wounded area (Reynolds et al., 1997; De Lima et al., 1999). Sprouted afferents appear capable of maintaining a larger number of functional synapses and exciting a greater number of cells than is normally the case (Torsney and Fitzgerald, 2003). This is more pronounced in neonatal than in adult wounding and is accompanied by hypersensitivity (De Lima et al., 1999). This mechanism could be one of the reasons for our finding, especially as we observed in our analysis of percentage change from the control area, that the neonatal scars were more sensitive than the older scars. It also appears that C and A δ fibres have a greater capacity for sprouting than low threshold mechanoreceptors (Kinnman et al., 1992) and we did not see any significant findings for touch perception in our group comparison.

Endogenous nerve growth factor levels are reduced in injured nerves compared to intact nerves, which correlates with decreased skin axon-reflex vasodilatation (Anand, 1996). This change of ability for vasodilatation could have an influence on thermal perception after injury.

When analysing the comparison between individual scars and their control areas we found that after scarring, regardless of the age when the scar was inflicted, there are almost always changes in perception thresholds in all the modalities we tested and these changes can result in increased or decreased sensitivity compared to the contralateral control site and again this can vary for the same scar for different modalities. All in all we found 3 trends: When testing for touch perception thresholds, in 87.5% the threshold was lowest next to the scar and in 50% highest on the scar; for warm perception in 56 % of scars the threshold was highest on the control side and for cold pain 62.5% of thresholds were highest on the scar. Although one has to keep in mind that these are trends, not significant results, if larger numbers of participants had been tested, these may have reached significance.

There could be a number of reasons for the variety of our findings: the tested scars were of variable size, some were barely visible, one or two had developed slight keloid, the scars originated from various different causes, some being superficial, some deeper and they were of variable age. Our number of subjects was also small. We considered increasing the number of testing sites, but decided against it as the experimental session already took up to 2 hours and participants reached their limits of concentration. It should be emphasized that most QST has been carried out in adults who have a much greater ability to concentrate.

It is possible that some of the results in this study could have been due to chance, but since there are almost no significant differences between groups in the NICU study, type I errors do not appear to have occurred. In addition, in the second study group (cardiac surgery group), where the scars were larger, involving more layers of tissue, and more standard, there were not only significant differences between groups, but the significance followed a consistent pattern. This further militates against the possibility

of chance findings/significance in the NICU group. However, interpretation from small samples should always be treated with caution.

Verdugo and Ochoa (1992) examined 465 individuals with disorders of the peripheral nervous system using a quantitative somatosensory thermotest. Their investigation yielded 13 different patterns of thermal hypoaesthesia or hyperalgesia. Looking at cutaneous sensibility after burn injury it was shown that deep burn injuries were more seriously affected than superficial burns (Malenfant et al., 1998). Zhang and Laato (2001) showed that in contrast to normal scars, hypertrophic scars were transversed by many bundles of axons. All this is proof that there are many confounding factors regarding the final sensory "outcome" of scar tissue.

Despite these confounding factors it is possible to consider broad mechanisms for altered perception. Again we would suggest that these mechanisms are more likely to originate directly at the site of injury or at the same segmental level of the neural circuitry, since we found changes in sensation at these body areas, but not globally. At the actual site of injury there is either the possibility of hypersensitivity which can be caused by hyperinnervation (Reynolds et al., 1997; De Lima et al., 1999) but there are also other factors to be considered, like synergistic actions of several chemical mediators, including potassium, hydrogen ions, 5-HT, histamine or bradykinin (De Lima et al., 1999). Diminished sensitivity could be caused by destruction of nerve endings, fibre loss of the injured site or incomplete fibre regeneration (Malenfant et al., 1998).

There is obviously also the possibility of more global changes in the central nervous system following these injuries and these might be so small that we were not able to detect them with our equipment, especially as they do not appear to impinge on daily life. Looking for more central causes of subtle change, one possibility is the expansion of dorsal horn neuron receptive field size, seen after skin injury at birth (Torsney and

Fitzgerald, 2003) and leading to prolonged behavioural hypersensitivity. Another explanation might be that altered patterns of C fibre excitation produced by local injury modify the activation of the NMDA and other receptor systems and with this mechanism change synaptic connectivity within the CNS (Fitzgerald and Walker, 2003).

Ren and colleagues report in their 2004 study two different patterns of consequences after neonatal scarring. The first is a localised enhanced hyperalgesia of the initially affected area emerging after a second insult later in life. The second shows a generalised reduction of sensitivity of the whole body. Looking at these two phenomena in context, Fitzgerald (2004) suggests that early pain experience enhances stress responses, which in turn increases stress-induced analgesia. Any local sensitisation that might occur at segmental level would be masked by this and require a strong stimulus to then be observed. In our study the NICU survivors did not have a detectable overall decreased sensitivity. Comparing neonatal scars with the contralateral controlsite however, we found in some scars the scar site to be more sensitive than the control site, in others it was the other way round. Definitely all our participants were subjected to some form of stress during their NICU stay, which though may not have been enough to cause a detectable hypoaesthesia, but may have been enough to mask hyperalgesia at the local scar sites.

Another point to mention is that when looking at sensitivity after scarring the age when the scar was inflicted (Fitzgerald and Walker, 2003) and the time passed after the initial insult (Malenfant et al., 1998) are two very important factors in regards to the consequences. There are so far no studies looking at human scars later than 18 months after injury (Malenfant et al., 1998). Our study is the first ever looking at a time span of 17 years.

2.4.3.2 Questionnaire

Looking at overall pain experience and perception, there was a difference between the two groups only in reporting amounts of worst pain intensities for common pains. Here the control group scored significantly higher. From earlier studies of Grunau and colleagues (1994, 1998, 2000) we might have expected to see greater differences, although no study has examined the age group studied here. However, we had a much smaller sample size than Grunau and colleagues and have to consider if our NICU survivor group was a typical sample of the overall population. Adolescents with bigger scars and possible worse experiences were sicker at the time and had subsequent long-term physical problems and cognitive deficits. They were excluded from the study as they met our exclusion criteria (hypoxic/ischaemic cerebral damage as diagnosed by ultrasound scan, neurological abnormalities on previous clinical examination, regular medication or drug use or chronic disease that could influence somatosensory function (e.g. Diabetes mellitus), and a WISC-R (Wechsler Intelligence Scale for Children) score of <80, indicating cognitive impairment (Roth et al., 2001). Our small sample was therefore a group that most likely experienced less pain in infancy than many other expremature NICU babies.

We recognise the limitations of the questionnaire used. This is the first time that a study of this kind on NICU survivors of this age has been conducted, and so we had to construct our own questionnaire with special relevance to the presence of long-term scars. Although we searched the literature for a suitable questionnaire, we could not find one. Therefore we took elements from a number of questionnaires that had all been previously validated, and combined these elements in our questionnaire. It is intended that validation studies will be conducted in future.

2.4.3.3 Material, Methods and other confounding factors

Sample of participants

Once we had been able to locate the current GPs of the participants we had a good response rate from GPs and also from the NICU survivors we contacted. We tested 6 NICU survivors and 6 control participants, which comprises a rather small sample and was mainly due to the difficulties in tracing the current GP's of possible participants.

Our sample was representative for very premature infants 17 years ago concerning their gestational age. We have, however to consider if our NICU survivor group was self-selective. We found only very small or no scars as a result of the NICU stay, which forced us to change our original plan of investigation. The question is whether this was the case because adolescents with bigger scars and possible worse experiences were sicker at the time and had subsequent long-term physical problems and cognitive deficits, which meant they had to be excluded from the study. This, in turn, would mean that the adolescents we tested may not have been a representative sample. However, a "representative sample " could not be tested with our testing methods as there would be too many variables for which we may not be able to control, as well as problems with cooperation and reporting, all of which would influence QST results.

All participants of our group were of the same age (17 years). In both groups we had no extremes regarding height or weight which could have influenced our test results (Meier et al., 2001). Both groups were matched for gender, which has an influence on touch perception thresholds (Bell-Krotoski et al., 1995). We aimed to match both groups regarding ethnic background and this was largely successful, although the influence of ethnic background upon pain assessment/experience is still not clear (Bernstein and Pachter, 1993).

We also hope to have accounted for influence of substances of abuse and analgesia, as we specifically requested these not to be taken and part of our questionnaire asked for these substances, but obviously in this matter we have to count on the honesty of our participants.

Environment

The testing room was the same for all participants, although our testing environment was not ideal as we did not have a soundproofed, air-conditioned room. The room temperature in our testing room was high, varying between 28 and 32°C. According to Meier and co-workers (2001) variations in temperature might influence the results for thermal testing, but as the temperature was similar in all cases, this should not influence overall comparisons between our two test groups or intra-individual comparisons. We tried to keep items in the test room always in the same position and minimize distraction and noise, although due to lack of soundproofing, noise outside the room was variable, and depended on the time and day of testing. Some of our participants arrived with their parents, and in 3 cases these attended the testing.

QST equipment, protocol and testing

Before starting to test our sample, we ran various trials on volunteers of a similar age, each tested by the same 2 investigators to confirm the consistency and reliability of our equipment.

The von Frey hairs we used were kept constantly in the testing room in the same conditions to avoid effects of changing environment on their accuracy, although we did not have any means of humidity control or measurement (Andrews, 1993). We strictly adhered to our algorithm and the filaments were applied as recommended by Bell-Krotoski (1990).

For thermal testing we used a small thermode size (18×18mm) as this thermode aligned best with the skin on our testing areas as recommended by Hilz and co-workers (1998). We had decided to use the method of limits as this is least time-consuming and our aim had been to test as many neonatal scars as possible.

In future studies, it would be interesting to investigate whether more subtle changes could be observed using a temporal summation protocol. It is possible that repeated stimulation might reveal an alteration in sensory processing, not shown to single stimuli. This was outside the scope of the current study. The entire testing session, including questionnaire and QST, was already lengthy, and the test subjects were adolescents and children whose concentration span was short. The protocol for summation can be long, but it would be worthwhile in the future, designing a shorter one for use in young participants.

The consistency of our results from the reference area both within themselves and with reference literature means we believe that we would have picked up any significant patterns of change.

Overall, the testing procedure was well tolerated. Nobody withdrew from the protocol and nobody reported severe discomfort or distress. Testing with von Frey hairs for perception threshold was generally said to be the most boring and most demanding on concentration, whereas testing for pricking pain with the electronic von Frey was deemed the most unpleasant. This test was also from the investigator's point of view the most difficult, as the equipment needs a lot of constant practice to apply linearly increasing pressure and to get comparable results.

For every testing procedure standardised approaches were used, including the wording of explanations, to make sure that there was no possibility of influencing the test results because of variability in the testing procedure or instructions. We thought this to be especially important as we had two testers.

As the graphs of individual results show, the standard deviations for pricking pain thresholds and cold pain thresholds are largest, making these two modalities the least reliable ones for comparisons. The reasons for this could be that the electronic von Frey hair was the most difficult equipment (see above), but also as the pricking sensation was the one deemed most unpleasant, it could be that the stress factor of the anticipation of experiencing this sensation may have resulted in a decrease of concentration. Cold pain is known to be a complex sensation, resulting from stimulation of A-delta and C-fibers with a range of receptors (Harrison and Davies, 1999).

Questionnaire

Before we used the questionnaire for participants, we tested it on adolescents of the same age, to see if they would understand the questions and were able to answer them, nevertheless we might have missed some specific points or expressions to get exactly the information we wanted.

Concerning the questions about the origin of scars it might have been better to have in every instance a parent, especially the mother present as they were the most knowledgeable ones.

2.5 Summary and Conclusion Chapter 2

We set out to look at neonatal scar sites and compare these with control sites in the same individual and with control sites in age-matched control participants. Contrary to our expectations, the neonatal scars in our cohort were scarce, on variable locations and small in size. As a result of this we tested a wide variety of scars of different ages and gained more general information about scarring rather than only specific information about neonatal scarring.

Despite our small numbers and scar variations, we have seen some trends and patterns but conclude they were not sufficiently large for a bigger study comprising NICU survivors with the same or similar degrees of scarring. We consider that using this methodology, we would have seen significant effects if they had been present.

Our study is the first to look at local sensation around a scar site after a prolonged period of time and we found an interesting variety of changes, which will stimulate many new ideas for further research.

Regarding our initial question about somatosensory changes in an area of neonatal scarring, we concluded that we needed to find a group of survivors with scars that are unilateral, clearly visible, and of equal size at the same segmental level. This will allow us to make clear intra- and interindividual comparisons.

Table 2.1:

Von Frey hair number related to stimulus intensity

Hair number (vFh unit)	Grams (g)	milliNewtons (mN)	% Increase
1	0.0174	0.1706	66
2	0.0292	0.2863	66
3	0.0479	0.4697	66
4	0.0794	0.7785	66
5	0.132	1.2943	66
6	0.219	2.1475	66
7	0.363	3.5595	66
8	0.603	5.9130	66
9	1.00	9.80	66
10	1.66	16.27	66
11	2.75	26.95	66
12	4.57	44.79	66
13	7.58	74.28	66
14	12.6	123.48	66
15	20.9	204.8	66
16	34.7	340.1	66
17	57.5	563.5	66
18	95.5	935.9	66
19	159.0	1558.2	66
20	263.0	2577.4	66

Table 2.2:

Thresholds in the Reference Area

	NICU survivors (n=6)	Control participants (n=6)
Touch perception Mean \pm SD	4.2 \pm 1.09 (von Frey hair number)	3.7 \pm 0.517 (von Frey hair number)
Cool perception Mean \pm SD	28.67 °C \pm 3.10	30.29 °C \pm 0.517
Warm perception Mean \pm SD	34.56 °C \pm 1.49	34.25 °C \pm 1.05
Hot pain Mean \pm SD	44.10 °C \pm 2.39	44.20 °C \pm 2.88
Cold Pain Mean \pm SD	15.57 °C \pm 2.24	18.18 °C \pm 5.43
Pricking Pain Mean \pm SD	349 mN \pm 142	385.5 mN \pm 85.5
Brush allodynia Mean \pm SD	0.112 (rating) \pm 0.274	0.83 (rating) \pm 2.04

Figure 2.1:
Von Frey hairs

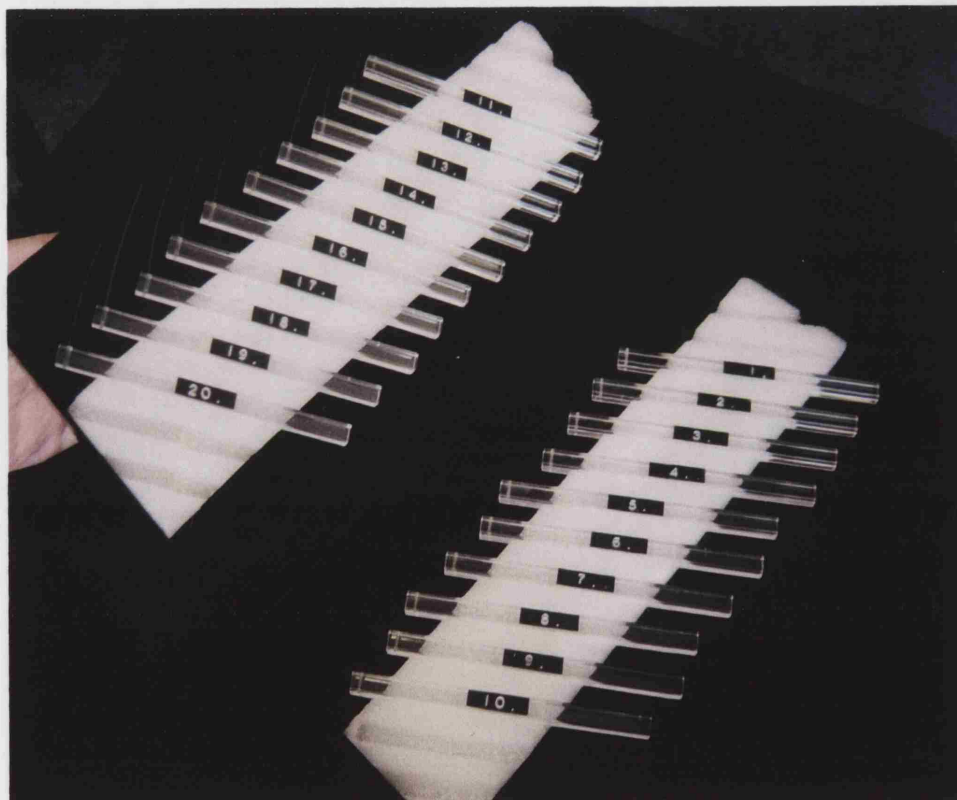


Figure 2.2:

Thermode with response button

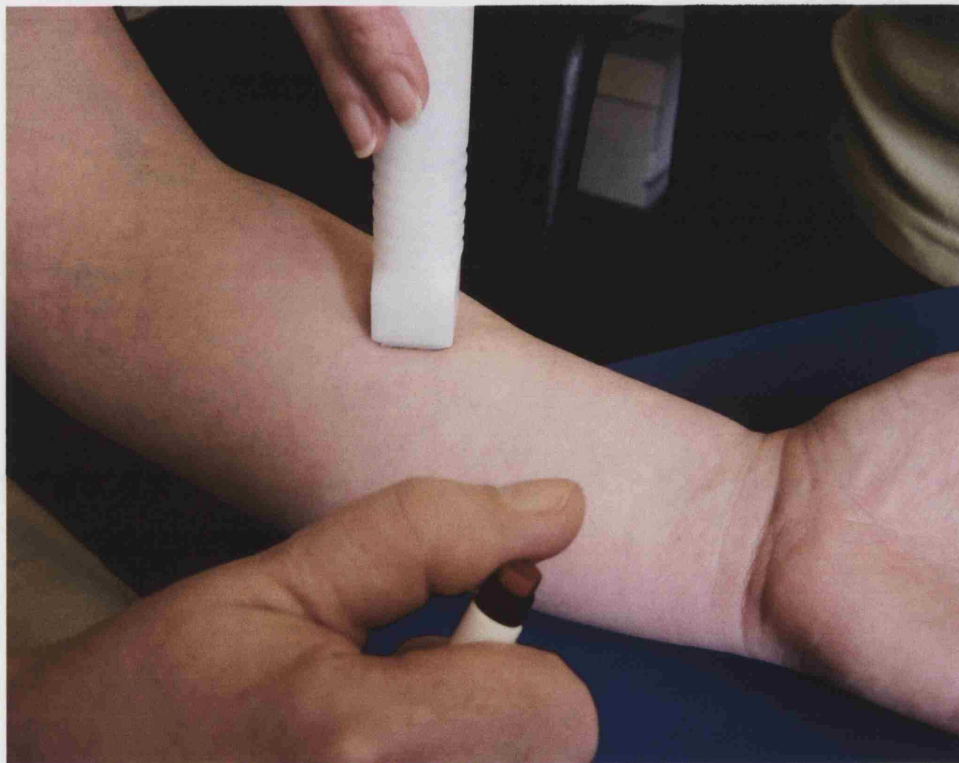


Figure 2.3:
Electronic von Frey

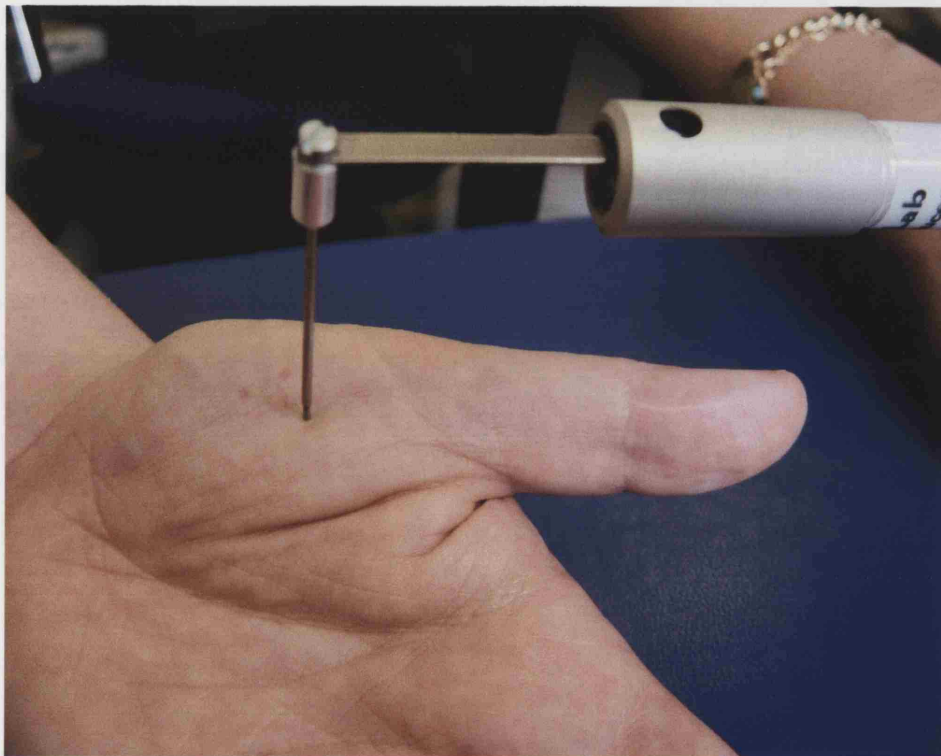


Figure 2.4:

Use of von Frey hairs

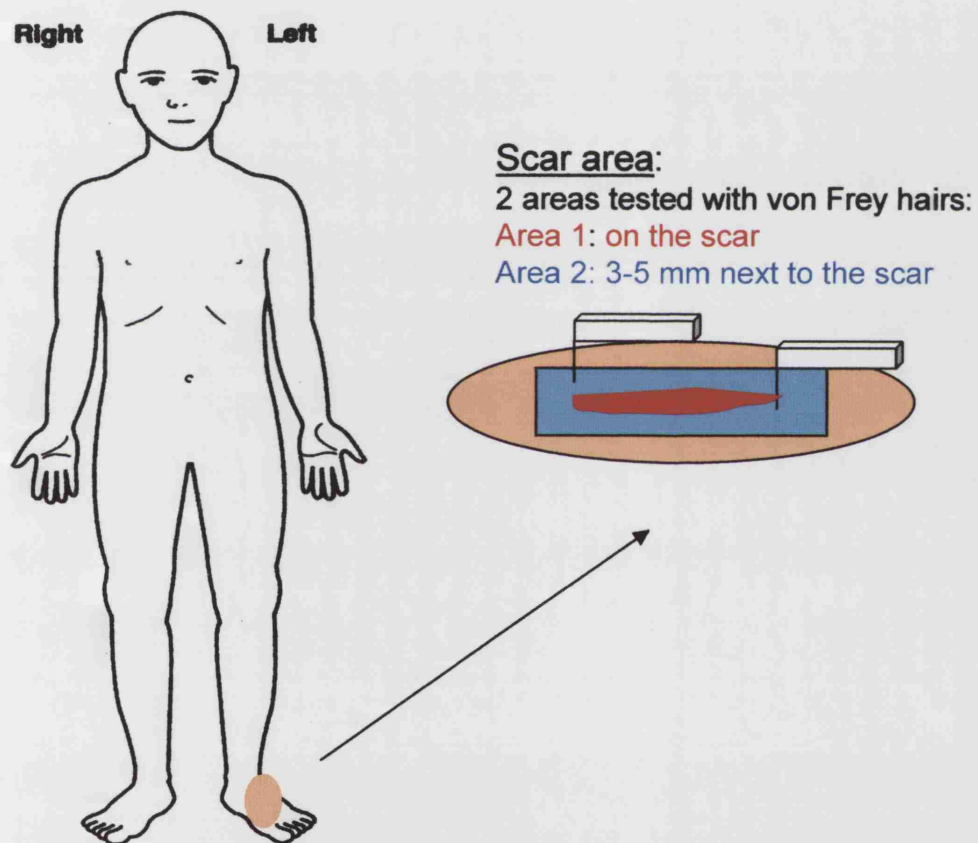
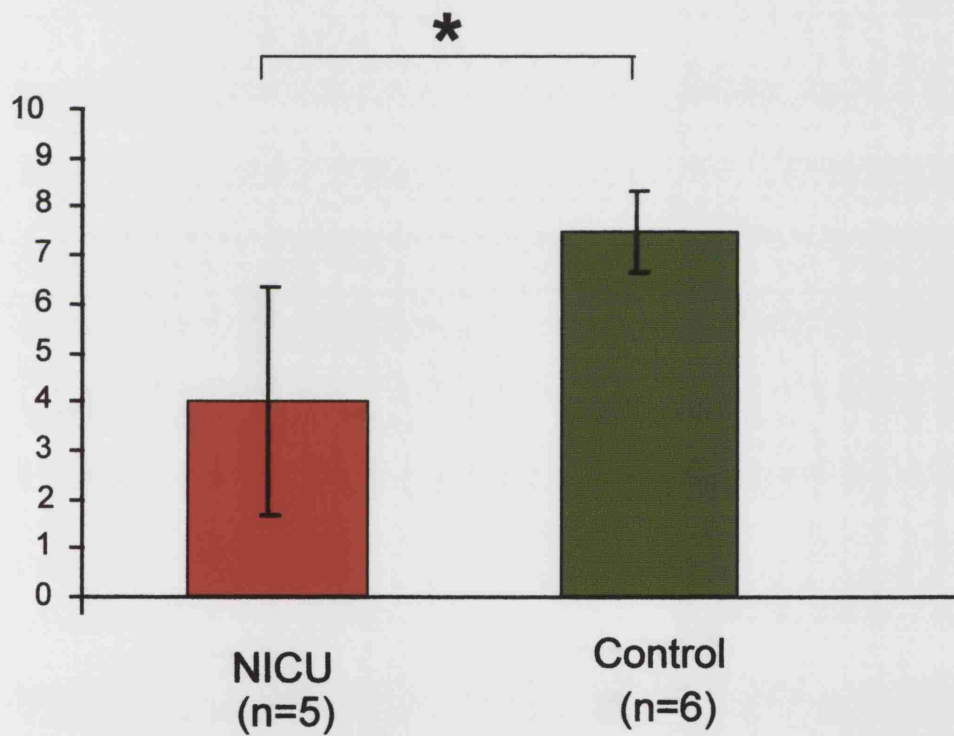


Figure 2.5:

Mean worst intensity pain is lower in NICU survivors than in control subjects



($p=0.034$, error bars denote SD)

Figure 2.6:

Location of scars tested in subjects of
NICU survivor group

X=older scar

n=definite neonatal scar

p=possible neonatal scar

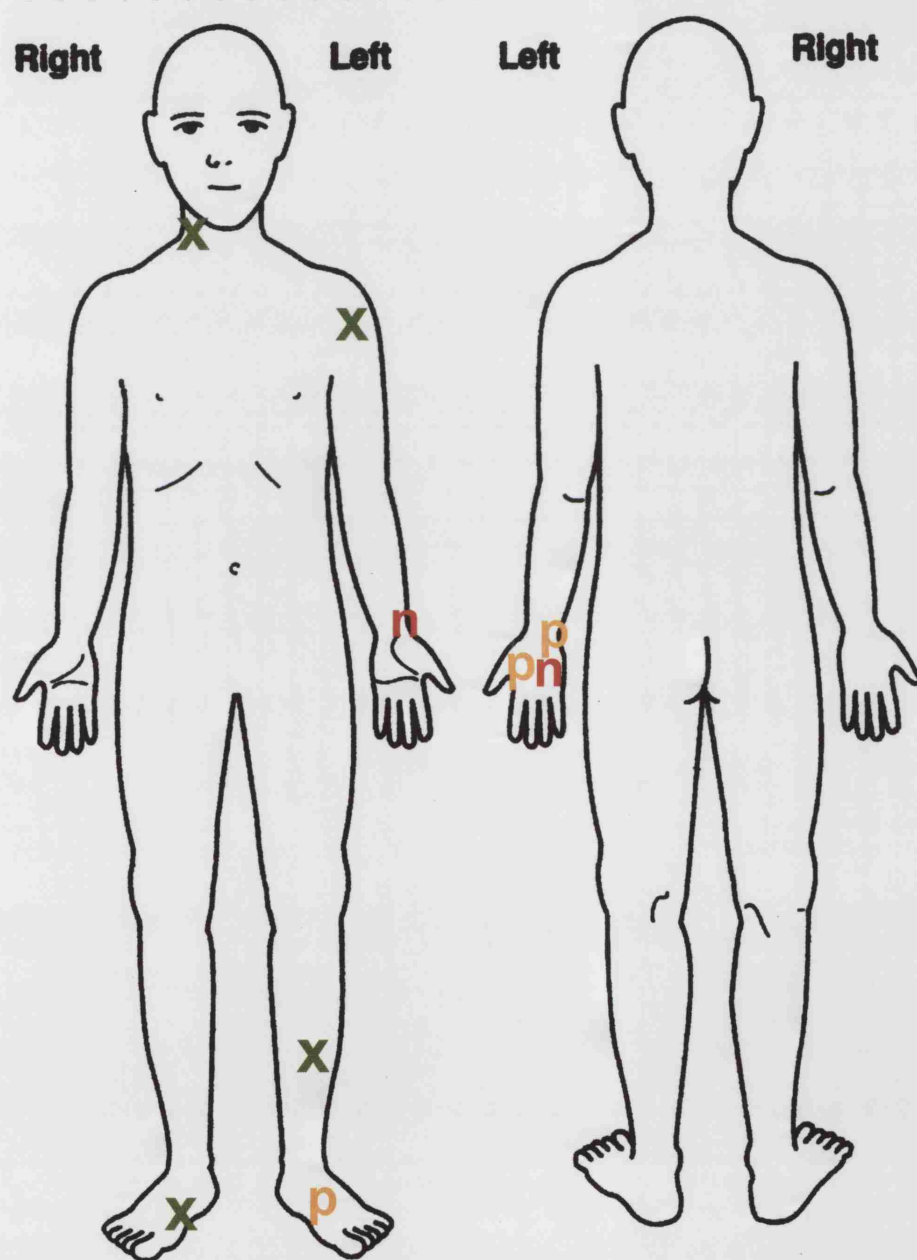
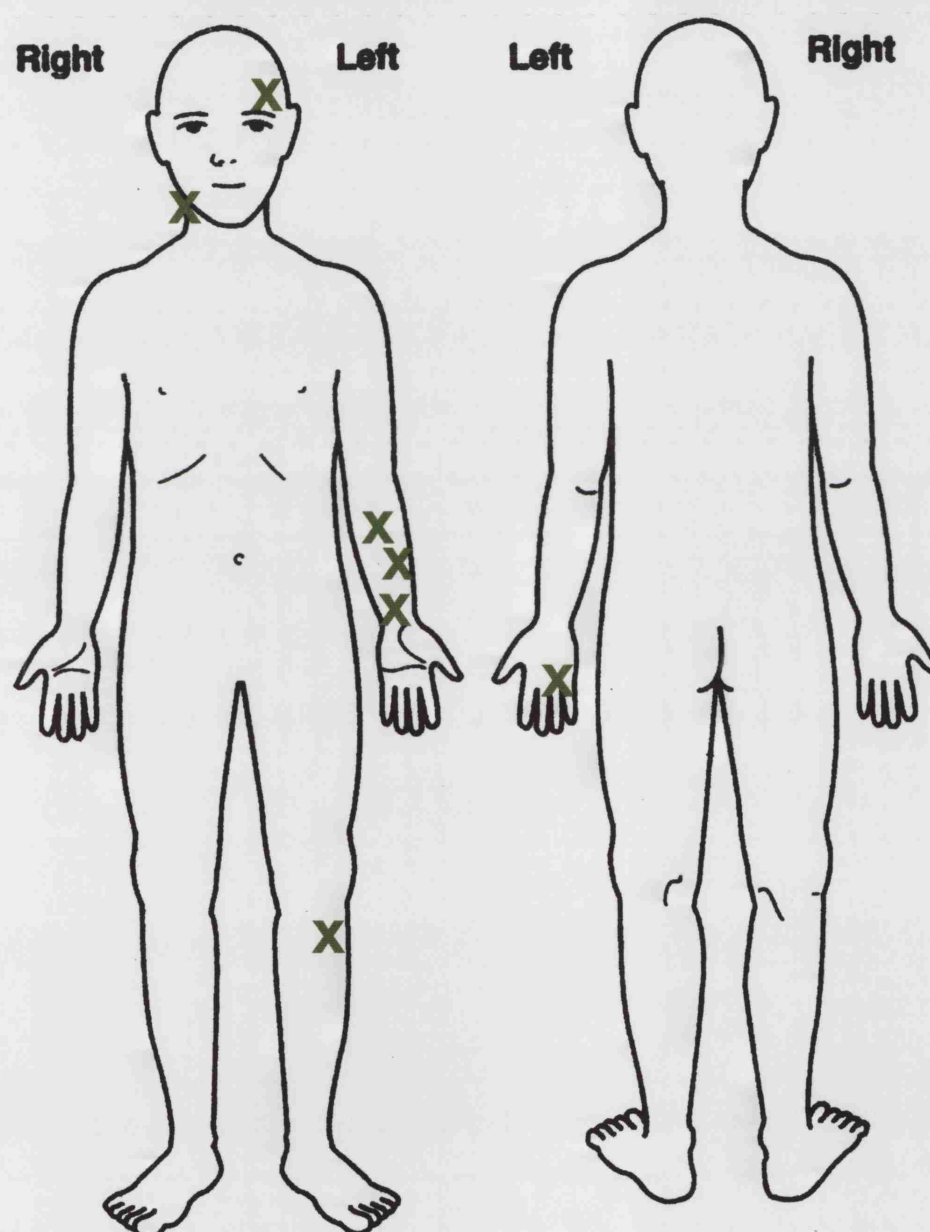


Figure 2.7:

Location of scars tested in subjects of control group

X=older scar



Changes in thresholds (%)

Contralateral control area threshold taken as 100%, result on/next to scar as percentage thereof

(Error bars show standard deviation)

Older scars Mean (n=11) Neonatal scars Mean (n=5)

Figure 2.8: Touch perception thresholds (%)

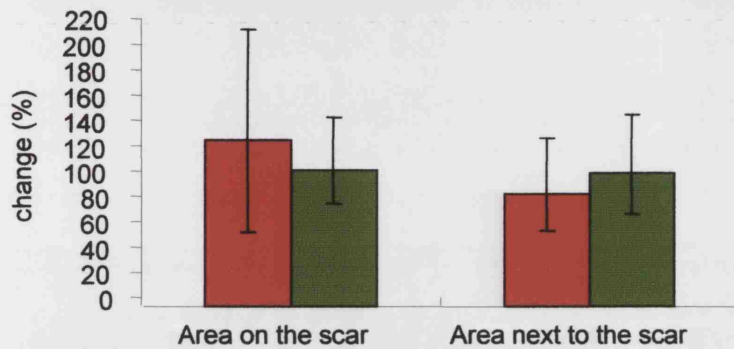


Figure 2.9: Pricking pain thresholds (%)

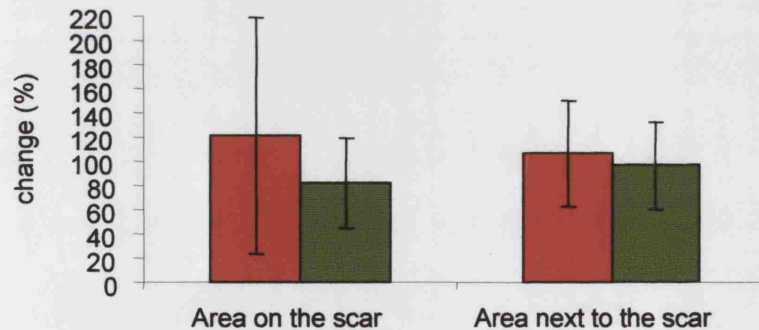
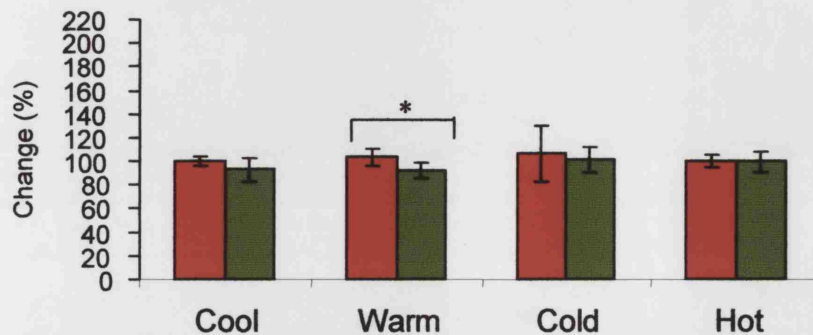


Figure 2.10: Thermal thresholds (%)



Frequencies of lowest and highest thresholds at scar sites and contralateral control sites in NICU survivors and control subjects

■ Neonatal scars (n=5) ■ Older scars (n=11)

Figure 2.11: Perception thresholds

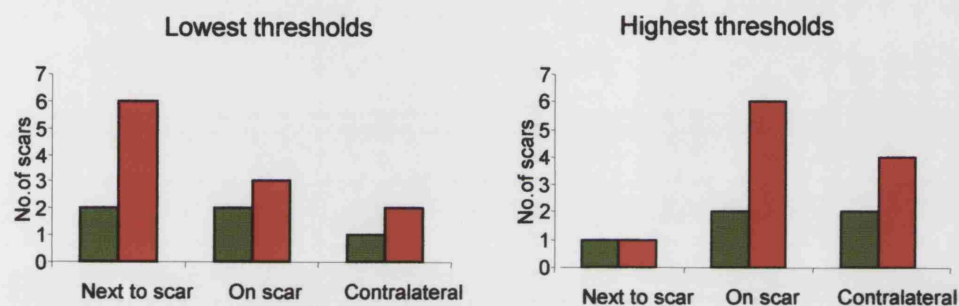


Figure 2.12: Pricking pain thresholds

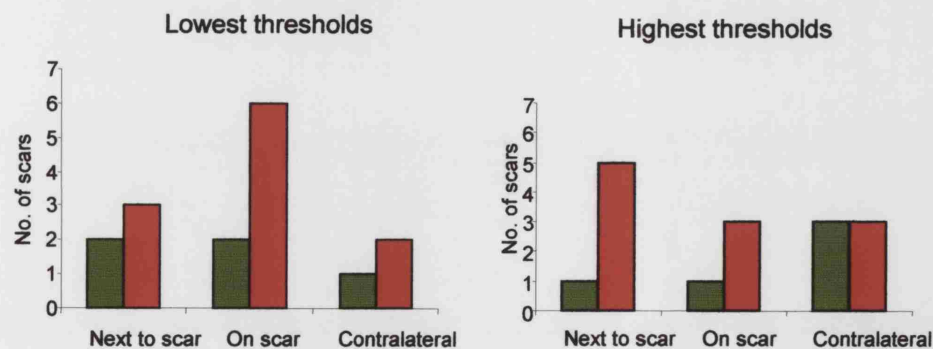


Figure 2.13:

Frequencies of lower, equal, and higher thermal thresholds at scar sites compared with contralateral sites in NICU survivors and control subjects

Older scars n=11

Neonatal scars n=5

■ Lower at scar site

■ Equal with contralateral site

■ Higher at scar site

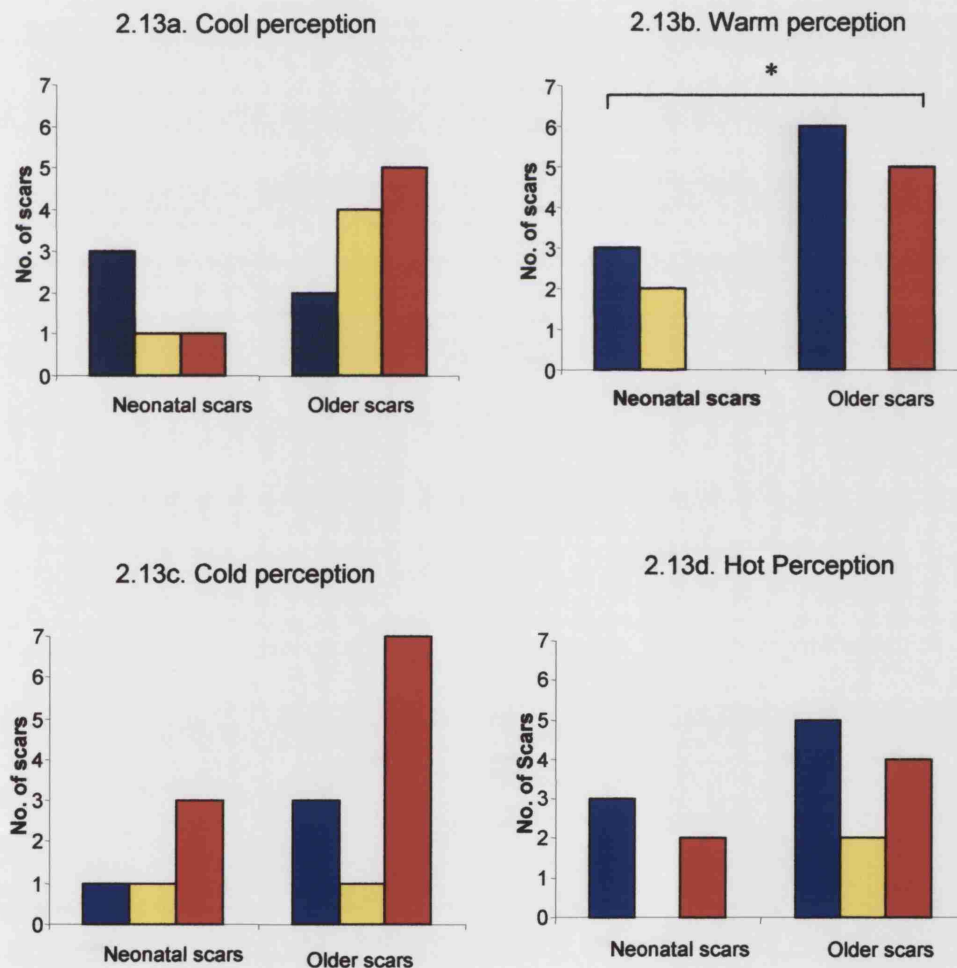


Figure 2.14 a-i: Touch Perception thresholds of all scars and tested sites individually

Sa = on scar Sb = next to scar C= control site

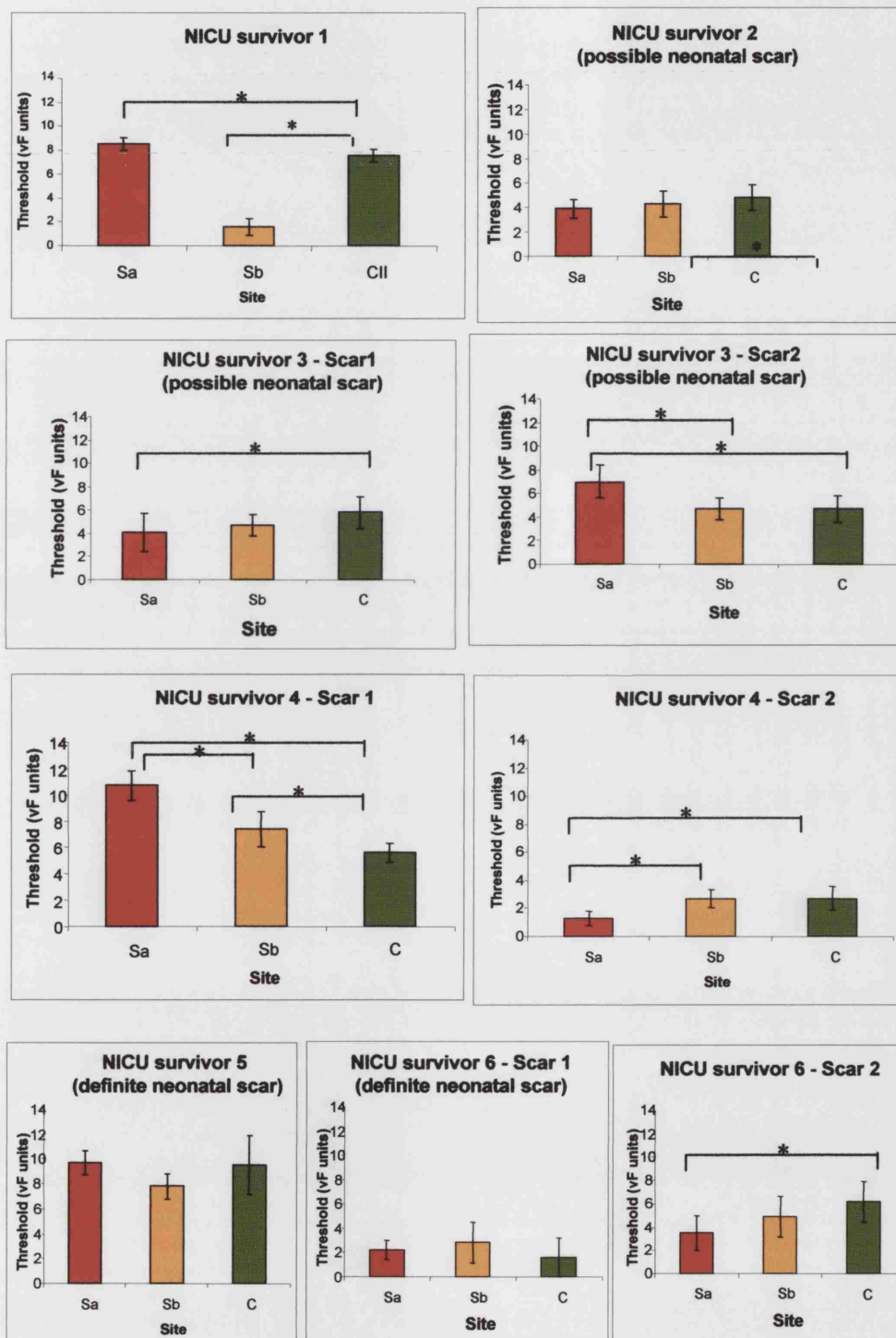


Figure 2.14 j-p: Touch perception thresholds of all scars and tested sites individually

Sa = on scar Sb = next to scar C= control site

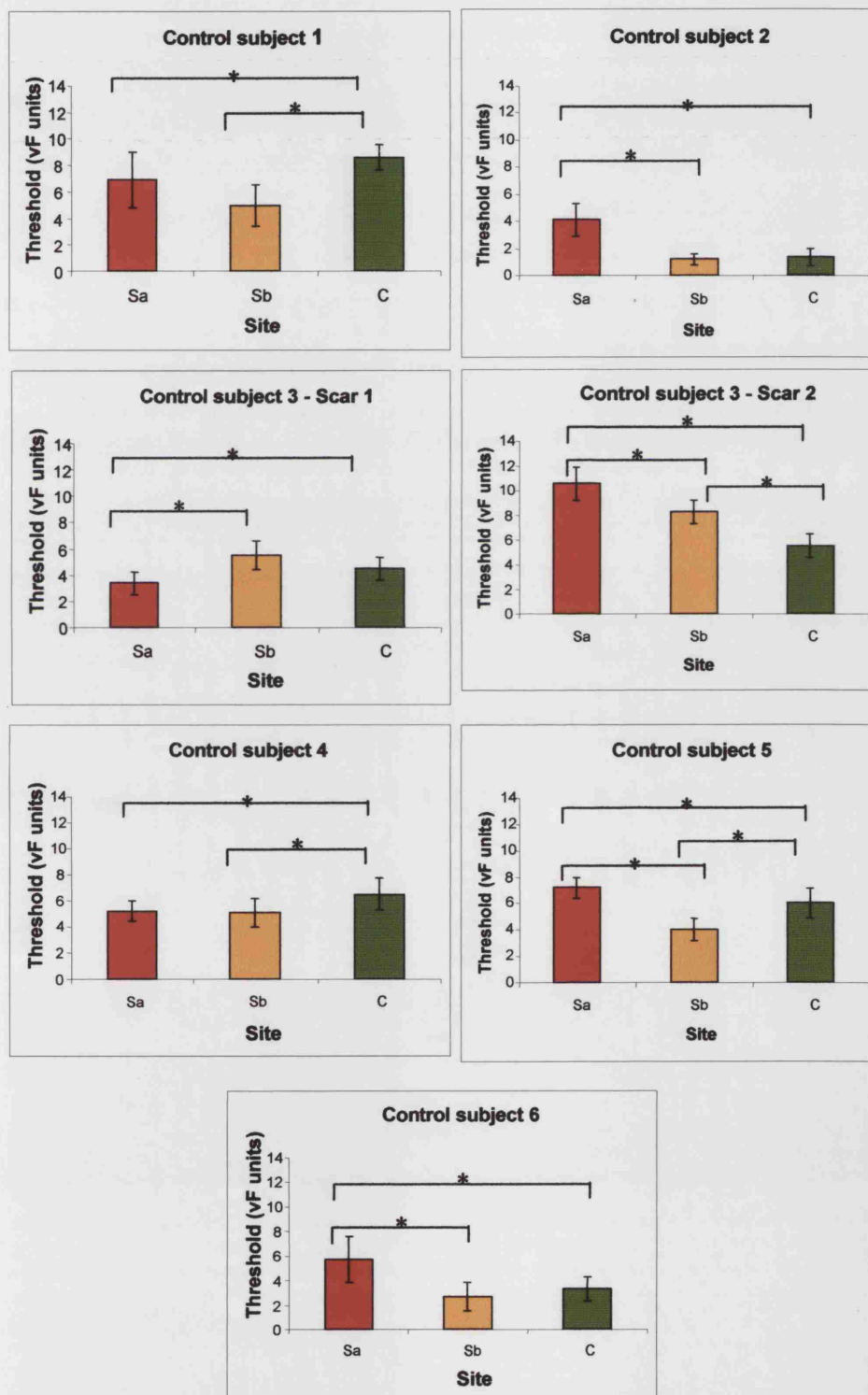


Figure 2.15:

Summary of location of highest and lowest perception thresholds in a scar area

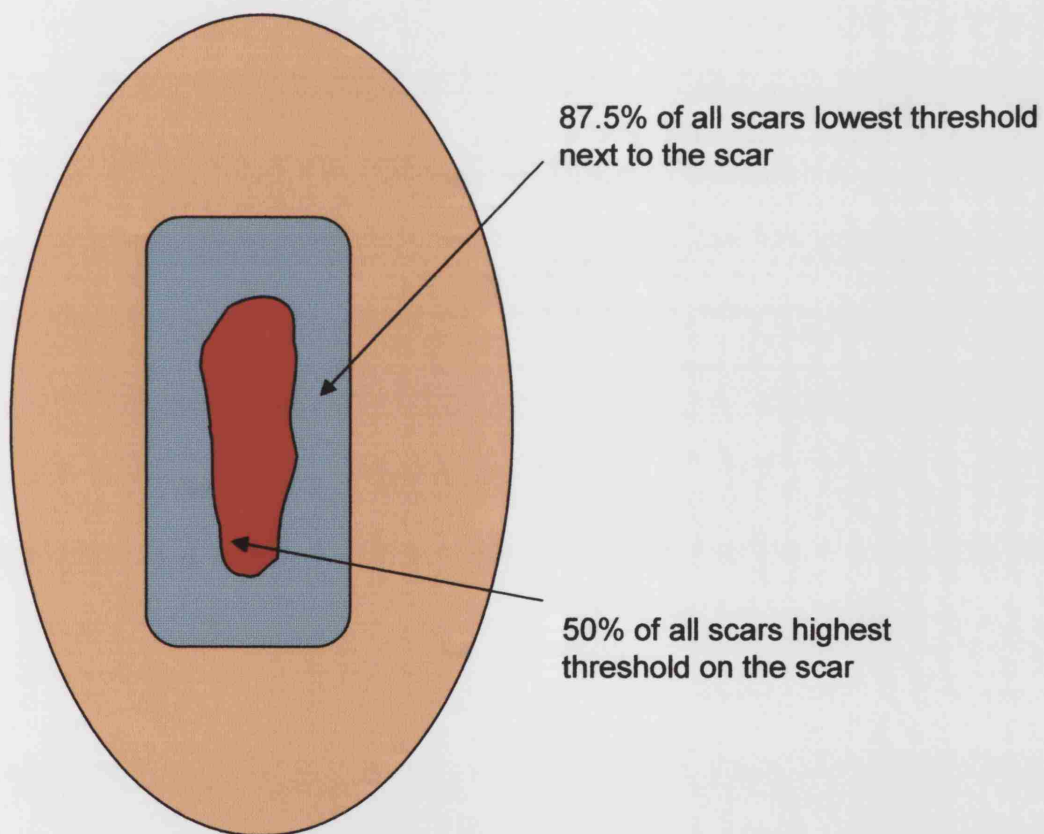


Figure 2.16 j-p: Pricking pain thresholds of all scars and tested sites individually

Sa = on scar Sb = next to scar C= control site

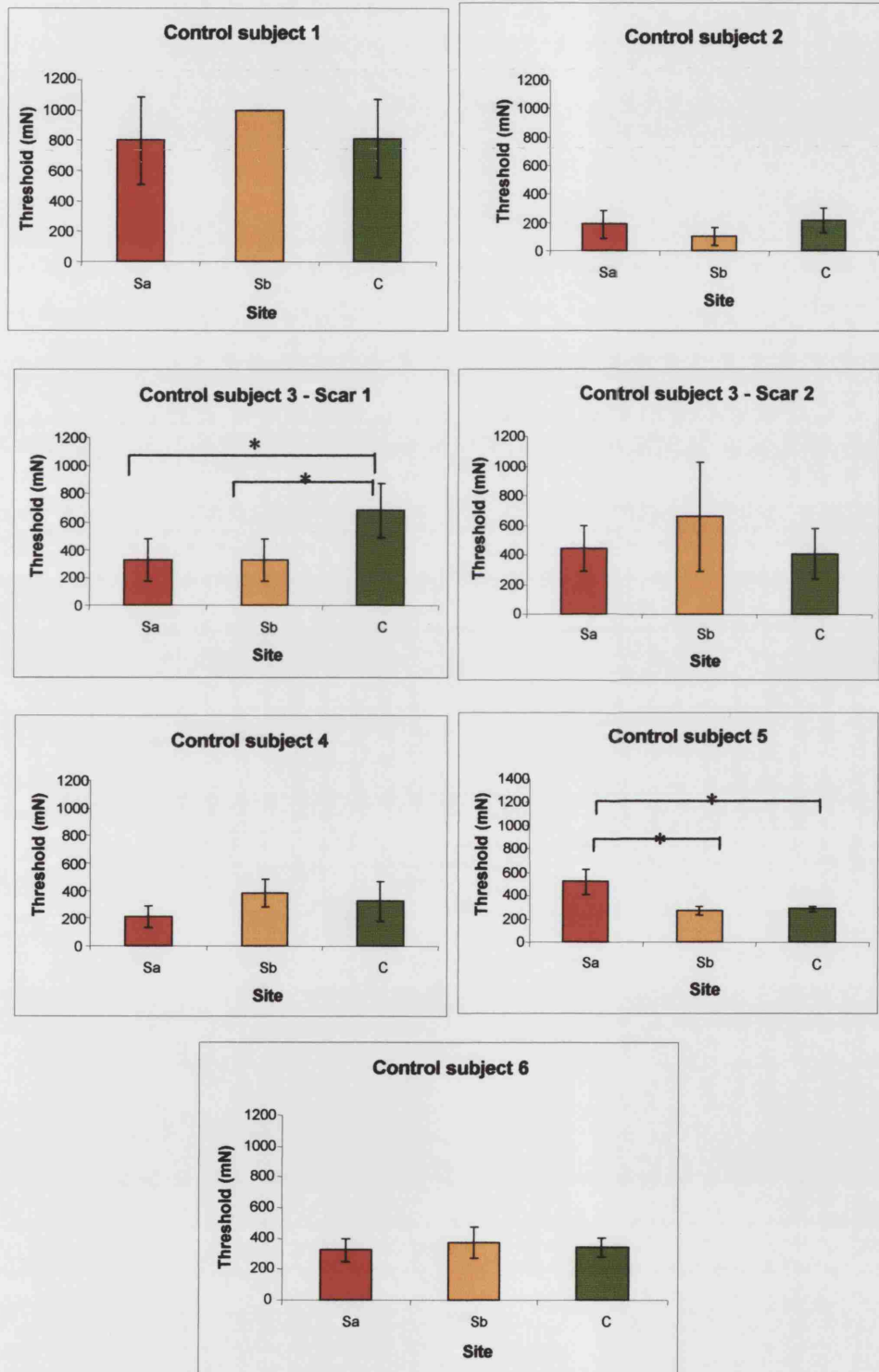


Figure 2.16 a-i: Pricking pain thresholds of all scars and tested sites individually

Sa = on scar Sb = next to scar C= control site

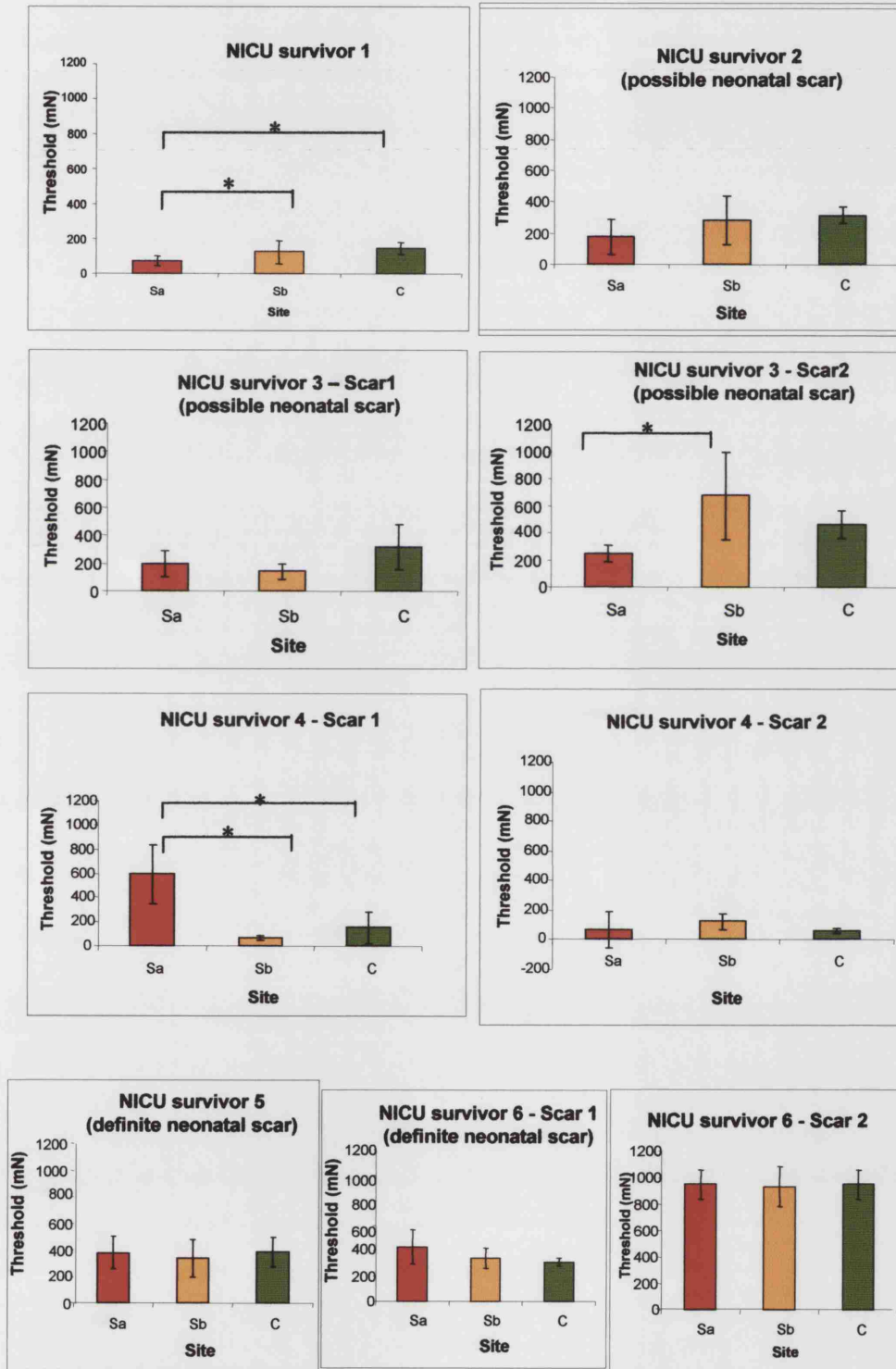


Figure 2.17 a-i: Cool perception thresholds of all scars and tested sites individually

S = scar C= control site

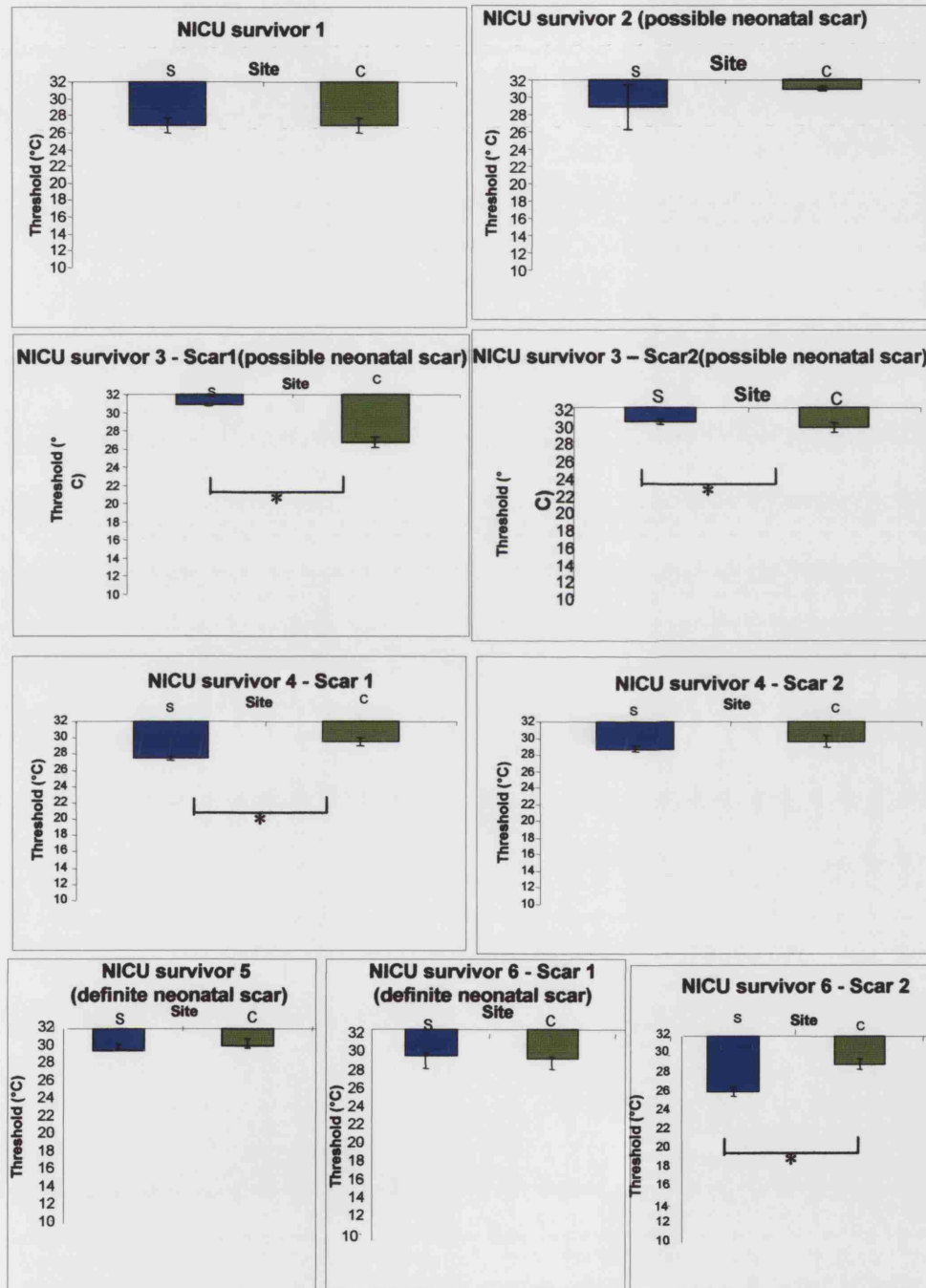


Figure 2.17 j-p : Cool perception thresholds of all scars and tested sites individually

S = scar C= control site

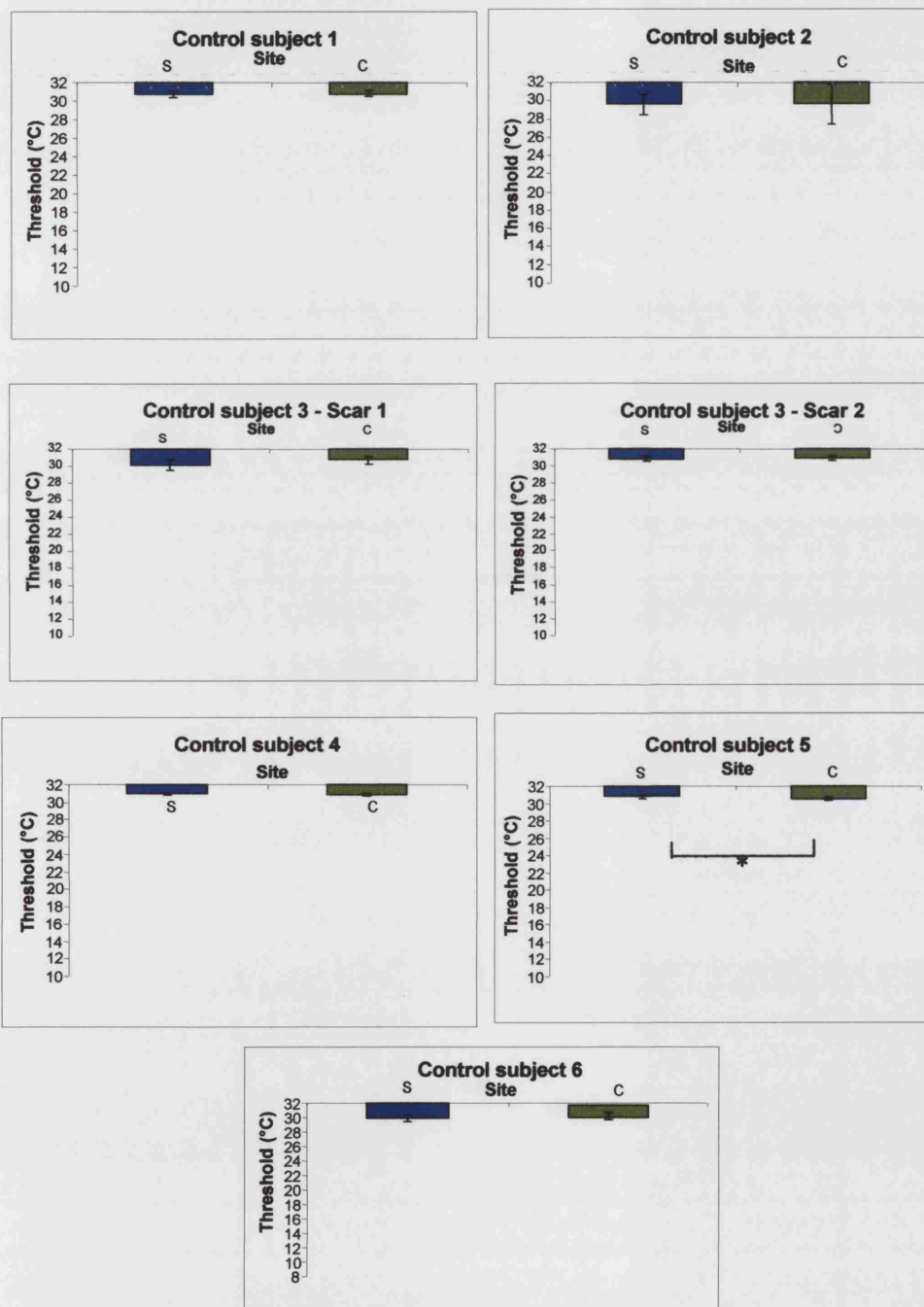


Figure 2.18 a-i: Warm perception thresholds of all scars and tested sites individually

S = scar C= control site

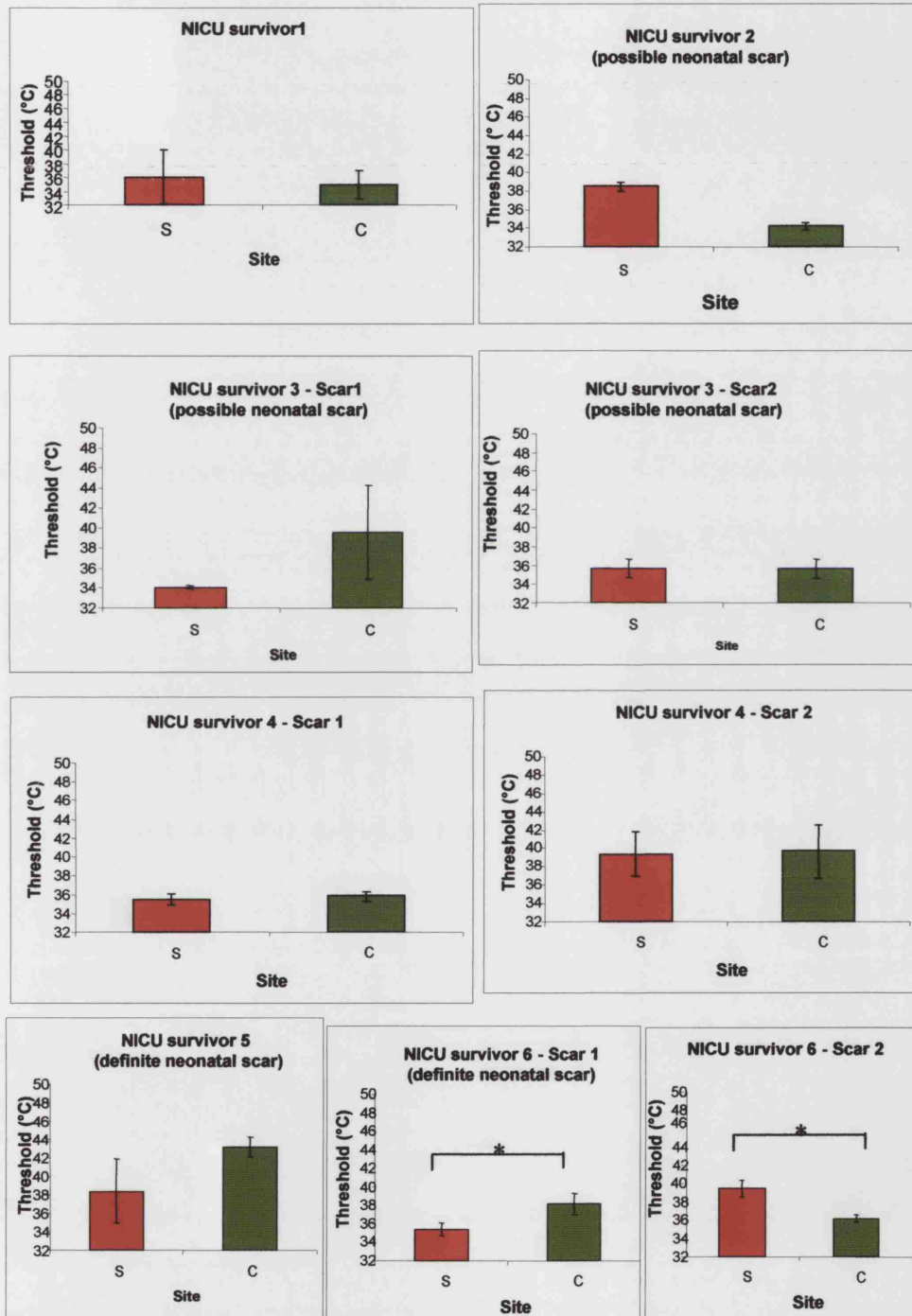


Figure 2.18 j-p: Warm perception thresholds of all scars and tested sites individually

S = scar

C= control site

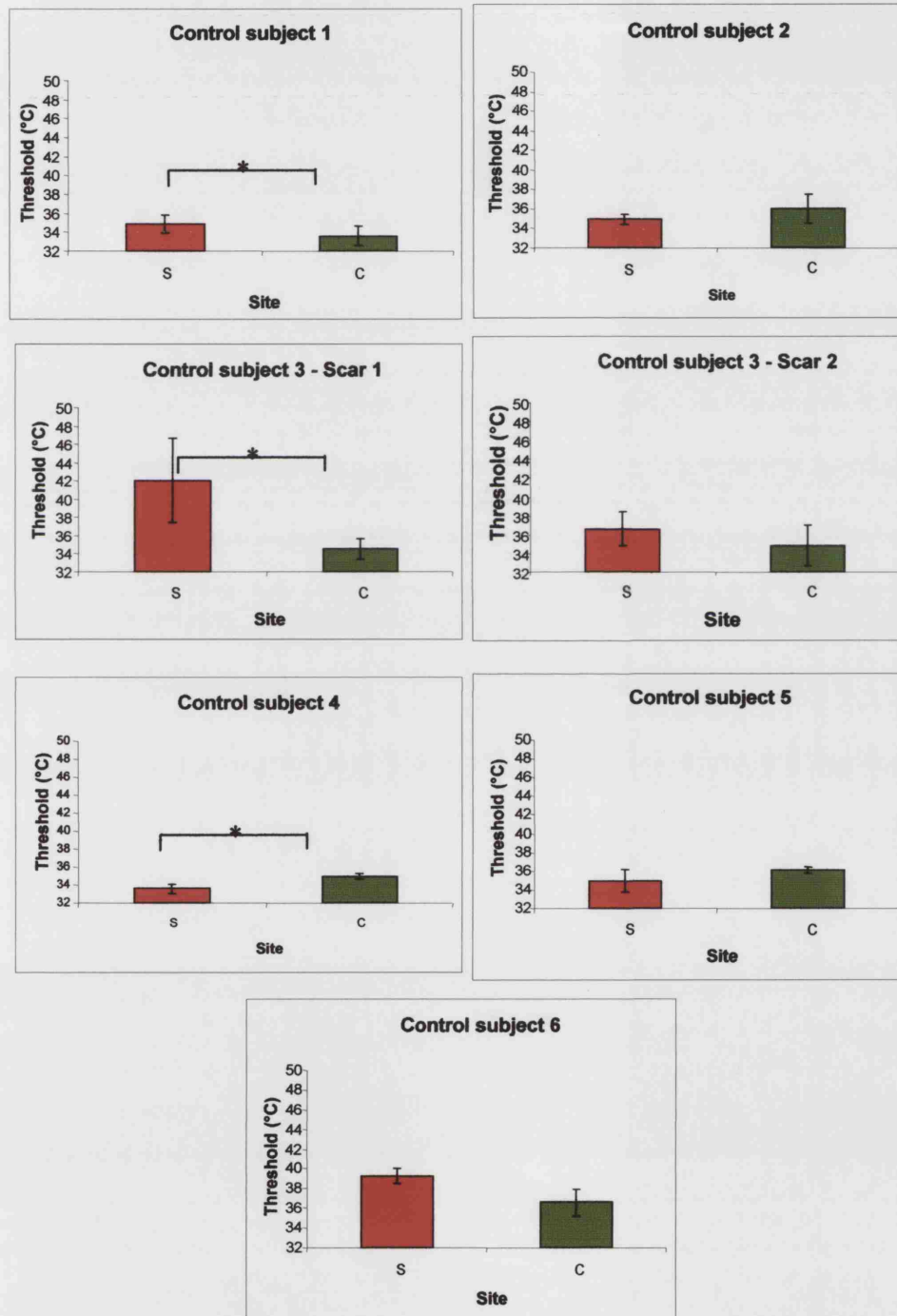


Figure 2.19 a-i: Hot pain thresholds of all scars and tested sites individually

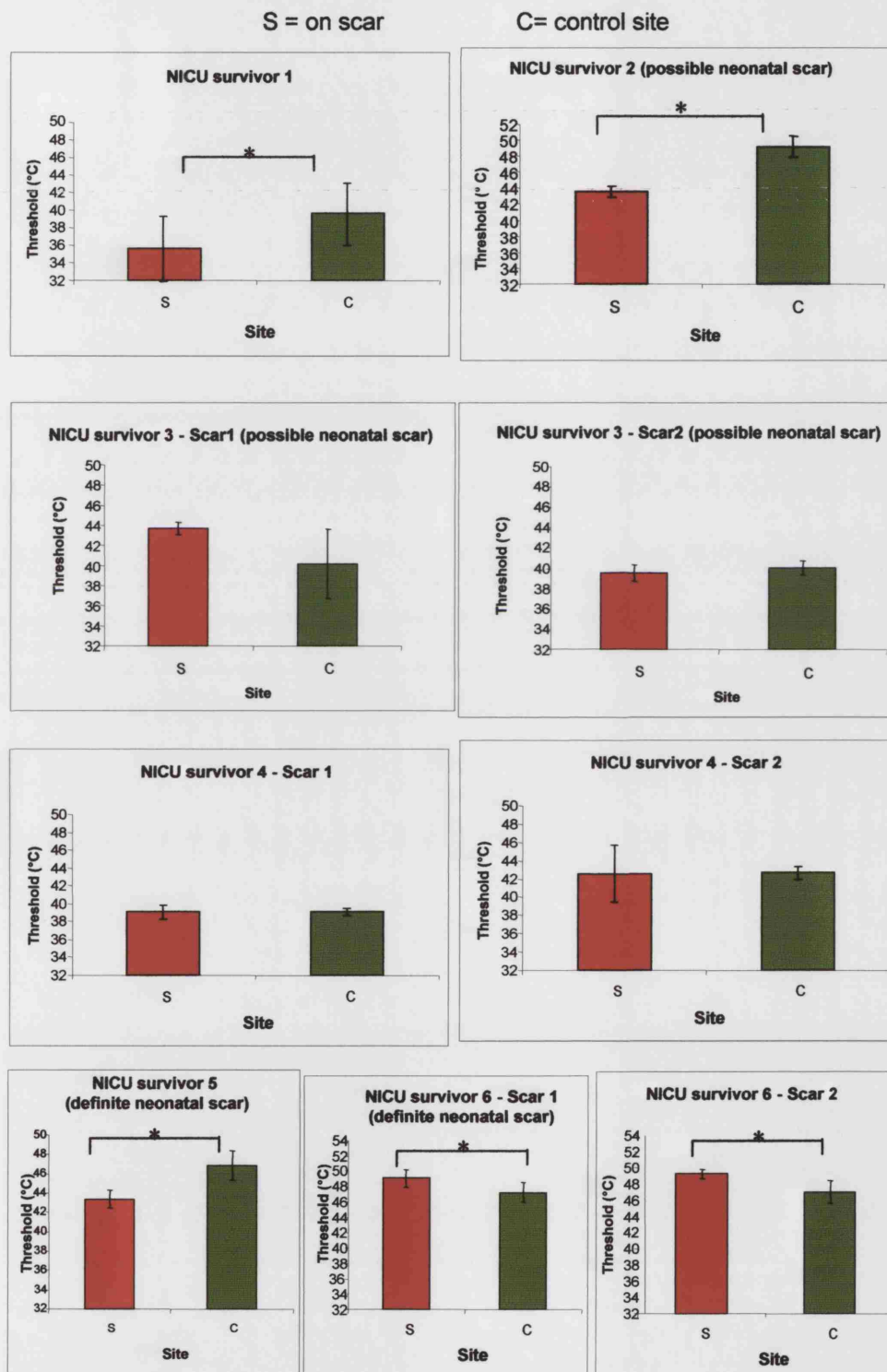


Figure 2.19j-p: Hot pain thresholds of all scars and tested sites individually

S = on scar

C= control site

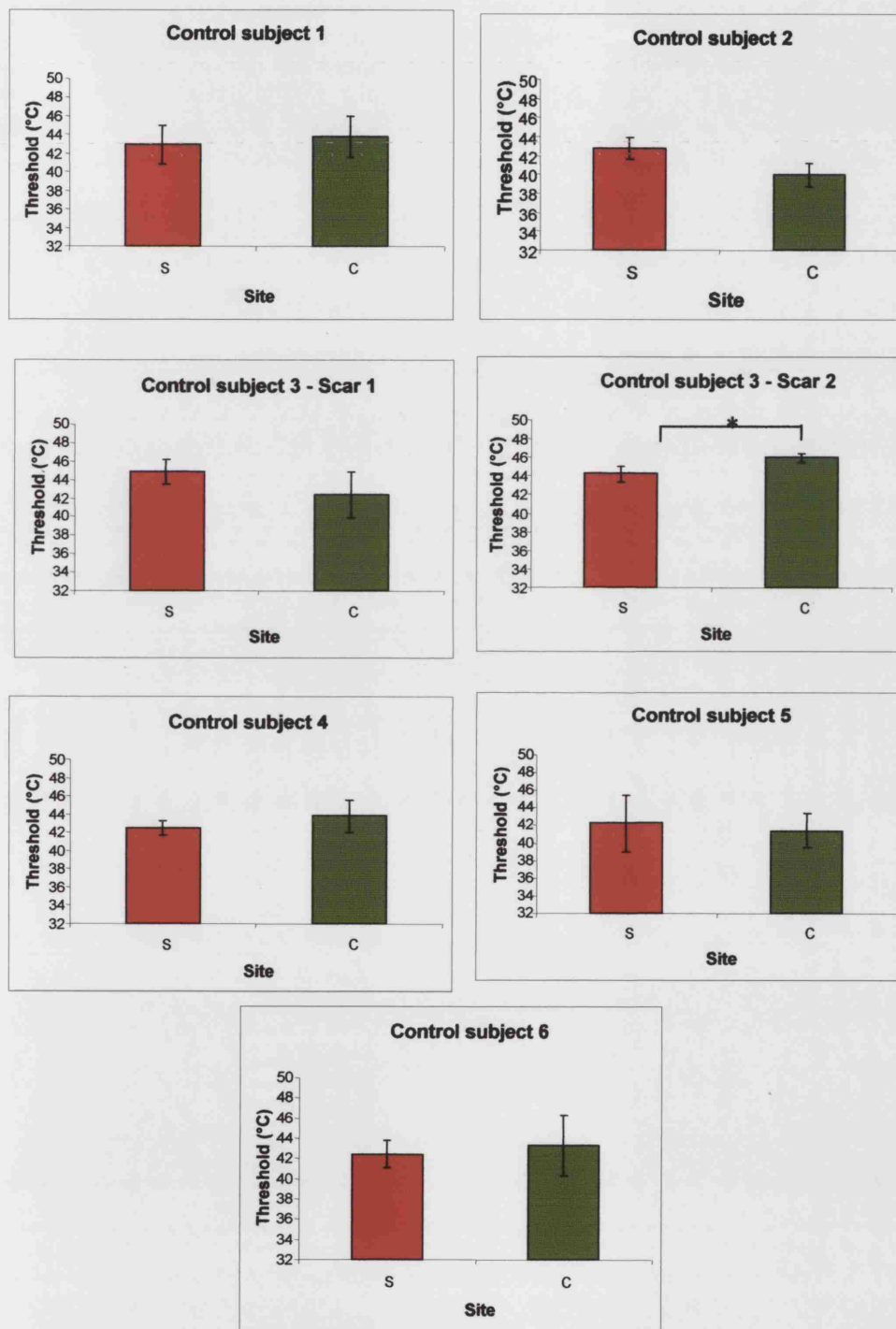


Figure 2.20 a-i: Cold pain thresholds of all scars and tested sites individually

S = on scar

C= control site

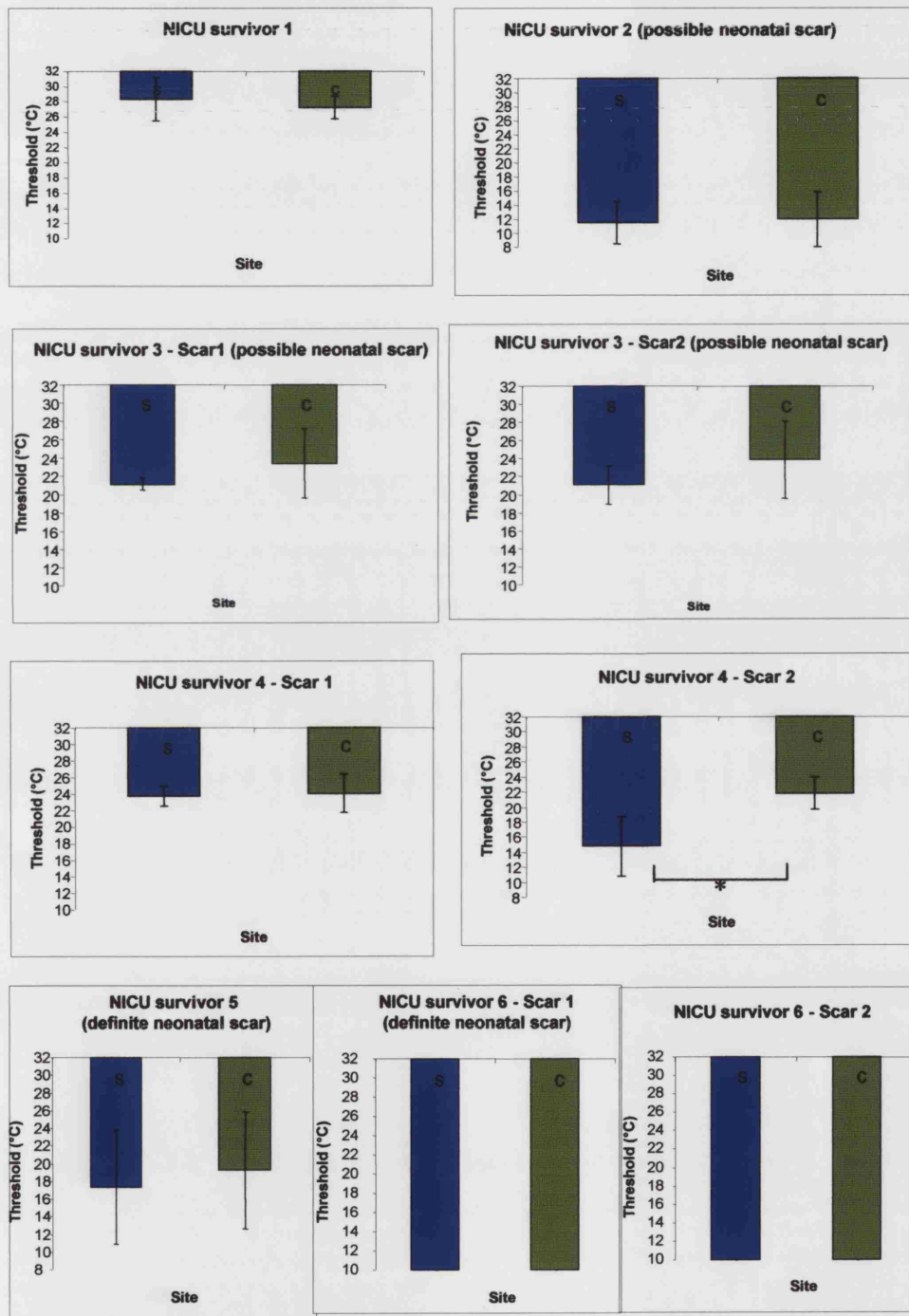
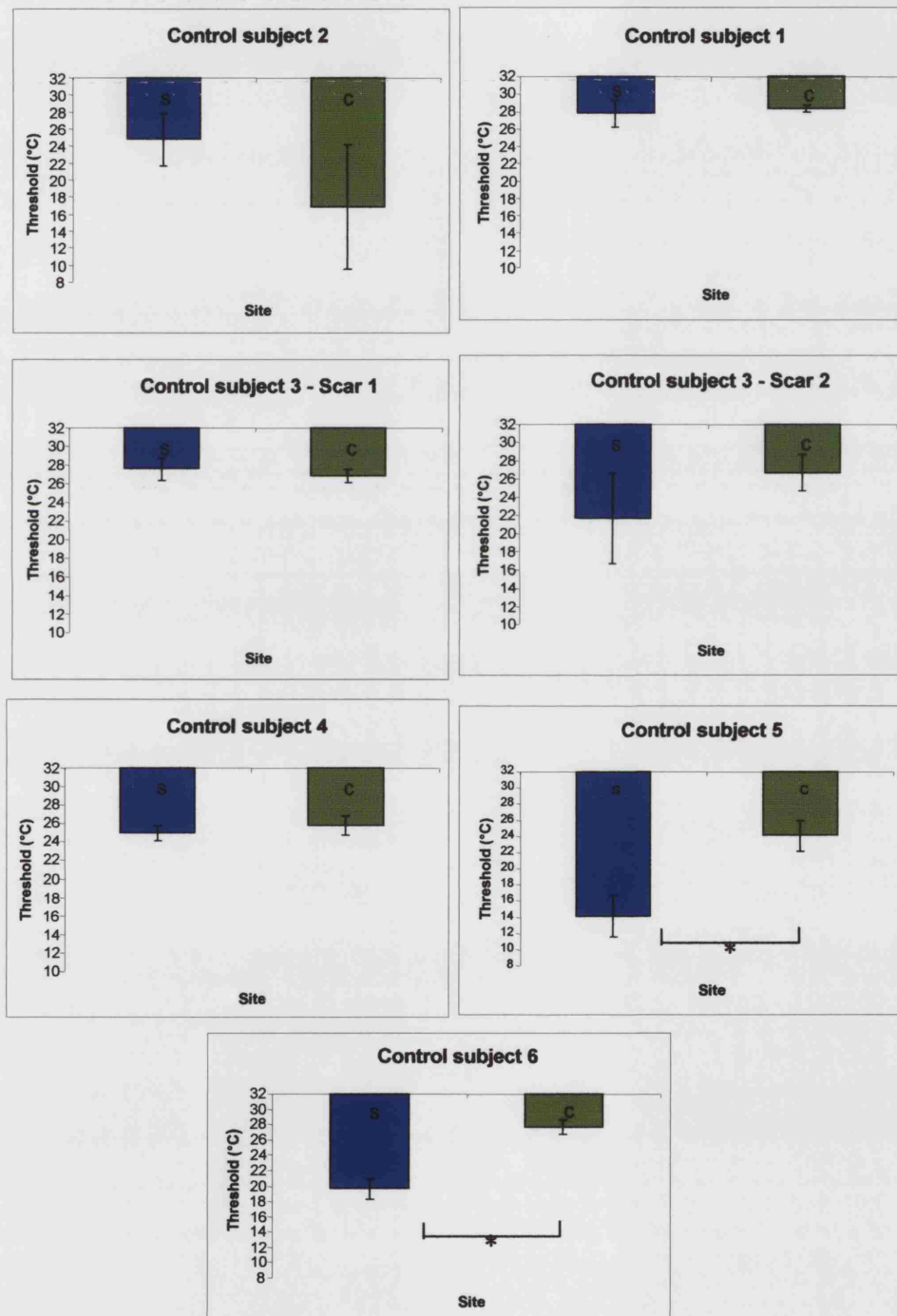


Figure 2.20 j-p: Cold pain thresholds of all scars and tested sites individually

S = on scar

C= control site



3. Cardiac surgery group

3.1 Introduction

The aim of this study was to investigate the long-term consequences of early infant injury using objective, reliable and repeatable measures. To do this we planned to use quantitative sensory testing (QST) on a group of participants that had undergone major surgery in infancy. Our objective was to test:

- (i) Scarred body areas, to measure long-term local effects in previously injured areas.
- (ii) Unaffected control areas of the body to provide a measure of background changes in sensory perception.

In the study described in the previous chapter we investigated neonatal unit survivors focussing upon QST in and around scars resulting from the treatment and care received as preterm babies. With this group we encountered problems with the actual scarring. Few of them had scars which they could trace back reliably as originating from their time in NICU. Scars of neonatal origin were very small in size and difficult to visualize, which made the testing procedure difficult. They were also on various different locations of the body or bilateral (cannula insertions), which did not allow for direct comparison of affected and unaffected areas.

Therefore for this study our requirements were:

- (i) An uniform injury of about the same size and location in each participant to allow for group comparison.

- (ii) An injury that was unilateral and not midline to allow for intra participant comparison.
- (iii) A resulting scar that was visible at an accessible site and of reasonable size for testing.

Further requirements concerning the participants were:

- (iv) An age group old enough to participate in QST.
- (v) Healthy at present and not on any medication that could influence somatosensory function.
- (vi) Able to come to our testing room with their parent or guardian.

We concluded that a lateral thoracotomy scar resulting from the repair of either a patent ductus arteriosus or a coarctation of aorta in the neonatal age group would fulfil all our requirements. This chapter describes the results from a group of 9-12 years old children with such scars along with age and gender matched controls.

3.2 Materials and Methods

3.2.1 Participants

Participants that had undergone a lateral thoracotomy in infancy were chosen as an experimental group for this study.

Surgery

Coarctation of aorta (CoA) (Figure 3.1): this is a constriction in the descending thoracic aorta in the region of the ductus arteriosus. Surgery in infants is most often

performed around the second week of life. This is generally the time when these newborn infants present with symptoms of cardiac failure.

Patent ductus arteriosus (PDA) (Figure 3.2): Especially in infants born pre-term the ductus arteriosus often remains patent, but this can also occur in term infants. Treatment is generally pharmacological at first using indomethacin, a prostaglandin inhibitor. Surgical ligation is necessary when this fails or is contraindicated. Therefore the timing of surgery is individual for every child, but generally, it is performed before six months of age.

Surgical incision

The surgical approach for both procedures is through a left thoracotomy performed usually in the fourth intercostal space. The patient is placed in a lateral position with the left side uppermost (Fig.3.3). The length of the skin incision depends on the particular lesion; it is shorter for PDA and longer for the full exposure needed for CoA, but in general about 2-3 cm. The incision can be straight or slightly curved. The skin is incised with a scalpel, and subsequent layers are opened with the point of diathermy (Figure 3.4). The following muscles are incised in layers: pectoralis major, latissimus dorsi, serratus anterior. Then the intercostal space between two ribs is opened with diathermy (Figure 3.5). Intercostal muscles and ribs are spread with an artery forceps and finally the pleura is entered. For closure heavy pericostal stitches are inserted, usually 3 to 4. Then the 2 muscular layers are closed, followed by the subcutaneous tissue and finally the skin. A drain is placed just caudal to the incision (Stark, 1994). It takes usually about 10-14 days for the skin wound to heal, the chest drain is generally removed after a few days.

Recruitment

The children with lateral thoracotomy scars were recruited by searching through the operating notes from January 1991 to December 1993 of the Cardiothoracic Department at Great Ormond Street Hospital. We included infants who had their operation within the first month of their life if born full term, or before the equivalent gestational age if born preterm. Their GP's were contacted to gain information about the possible participants as in the previous group (Appendix 3.1). Following the GP's response the medical notes were screened for exclusion criteria as before.

The parents of the participants were then sent an invitation letter and information leaflet about the study together with a reply sheet (Appendix 3.2). Once the reply sheet had been returned the parents were contacted, further questions discussed and the appointment confirmed. Control subjects were recruited from friends of the children of the researchers. At the testing appointment, verbal consent was obtained from the children and written consent was obtained from the parents including permission for photography of the scar. The children were made aware of their right to withdraw permission for the continuation of all or any portion of the tests. The parents were reimbursed their travel expenses and the children received £20 for participating in the study. All testing was conducted from 24.07.2003 to 31.10.2003.

3.2.2 Procedure

The study protocol was approved by the Joint University College London/ University College London Hospitals Committee on the Ethics of Human Research and also by the Chairman of the Great Ormond Street Hospital for Children NHS Trust/Institute of Child Health Local Research Ethics Committee.

After familiarizing the participants with the situation and obtaining consent, the investigator went through the questionnaire, which was slightly modified from the previous version (see below). After asking questions about the thoracotomy scar, this was measured and a picture was taken if permission had been given.

QST was done in the same quiet, draft free room as before. The participants were in sitting position for testing the reference area and were lying on their sides for testing thoracic areas. They were asked to keep their eyes closed during testing and were not given auditory cues to indicate the start of a stimulus. All were given the same instructions and a short demonstration before each type of test to familiarise themselves with the test. Also, the parents were offered a demonstration of each test on themselves to alleviate any anxiety about the nature of the testing.

Each participant with scar was tested on (Figure 3.6):

1. the thenar eminence (reference area)
2. the lateral thoracotomy scar site (left side of thorax) and
3. at the corresponding site on the contralateral side of the body as intra-subject control (right side of thorax).

Each control participant was tested on:

1. the thenar eminence (reference area)
2. at the two same sites of the thorax as the scar group

The same standardized testing procedures were used for reference area, control site and scar site. These 2 groups of participants were tested for touch perception threshold with von Frey hairs, warm and cool perception thresholds, and brush allodynia. They were not tested for pricking pain, hot pain, and cold pain thresholds for three reasons. Firstly, inflicting pain at this age group would lessen the degree of

cooperation and make recruitment more difficult. Secondly, the pricking pain device was unreliable and difficult to apply, and thirdly, the results for cold pain had been rather inconsistent. The most consistent results in the first study group were with cool and warm perception thresholds.

Before each series of tests the room temperature was recorded.

3.2.3 Questionnaire

Compared to the previous group of NICU survivors, the participants in this thoracotomy scar group were only asked detailed questions about this specific scar and the control group participants were not asked about scars at all. In the section about general pain, the question about pain due to bruising was removed as participants from the previous group found this difficult to answer, and the question concerning period pain was removed due to the age of the participants. This age group was asked about taking pain relieving substances but not about substances of abuse. When discussing aspects of the thoracotomy scars, photographs of these were taken.

3.2.4 Apparatus

Mechanical perception threshold was tested with the same calibrated set of von Frey hairs as in the previous group. Thermal perception thresholds were tested using a 18x18 mm (3.24 cm²) contact thermode. Finally, brush allodynia was tested using the same Senselab brush as previously.

3.2.5 Testing Algorithms

All participants were tested on the reference area to begin with, then half of the scar group was tested first on the scar site followed by the contralateral control site, and the other half of the group was tested first on the contralateral control site, followed by the scar site. In the control group, half were tested first on the right site of the thorax and half first on the left. In this way any possible influence of testing order on the results was excluded.

Modalities tested on each site were:

1. Mechanical perception threshold with von Frey hairs
2. Cool and warm perception thresholds
3. Brush allodynia.

The testing algorithms for each modality were kept the same as before.

3.2.6 Statistical Analysis

For statistical analysis the same software as before was used and the same principles applied concerning von Frey hair numbers.

Group data

Analysis of the effects of site (reference, left and right thoracic), group (scar or control), and individual participant effect, on thresholds for all modalities (von Frey perception, cool and warm perception), was performed using nested univariate

analysis of variance (ANOVA), after first checking assumptions of normality. The interaction between site and group was also examined within this ANOVA. The model for the analysis was as follows:

Site ! Group Subject(Group)

Post hoc comparisons of all pair wise combinations within and between groups were made using the Tukey method, since it is the most conservative method for this kind of comparison, and there is least likelihood of producing a false difference between groups (Type I error, Hsu 1996).

Individual data

For each subject individually the data from all three sites were analysed for touch, cool and warm perception using a single factor (site) ANOVA after first checking assumptions of normality, followed by Tukey's pair wise comparisons.

Questionnaire

The differences between scar and control groups in the numbers of pains experienced and the amount of worst pain from these pains were compared using the Mann-Whitney test for unpaired observations.

For all statistical tests, $P \leq 0.05$ was considered statistically significant.

3.2.7 Role of MD candidate in the execution of the project

For this second project the candidate had to obtain ethics committee approval from University College London and Great Ormond Street Hospital. She recruited participants (without secretarial help), designed the new final QST testing algorithm and adjusted the questionnaire. The testing equipment was set up again and made

operational by the candidate. Testing itself was conducted by the candidate with 2 participants being tested by Dr Katharine Andrews Campbell. Some pilot testing sessions were carried out together in order to ensure that both experimenters used the same terminology and experimental methods. Some pilot participants were tested by both experimenters blinded and results compared to ensure as little variation as possible between experimenters. The candidate analysed the questionnaire and QST results single handedly and the statistical analysis was undertaken under Dr Andrews' supervision.

3.3 Results

3.3.1 Recruitment of subjects

50 suitable participants who had had an operation in the neonatal period (or before 44 weeks gestational age if born preterm) requiring a lateral thoracotomy were found in the review of operating notes from 1991-1993 in the Cardiothoracic Department at Great Ormond Street Hospital. The GP's of these children were contacted and 36 responded (72%). For 6 children the GP's did not feel it would be appropriate to contact them, the main reason being complicated family situations. A further 3 were excluded due to additional health problems, which left 27 children who received an invitation letter for the study. 10 families (37%) responded, 1 did not arrive for their testing appointment, which left 9 children who were tested.

3.3.2 Demographics of participants

From the scar group 3 children were born preterm, one at 26, one at 30, and one at 35 weeks gestational age. These children had surgery for a PDA at 31, 33 and 44 weeks gestational age. The child born at 30/40 weeks had also surgery for necrotising enterocolitis. The child born at 26/40 weeks had also surgery for hernia repair and was ventilated over a prolonged period of time; he spent 4 months in hospital before discharge. The last of these 3 children had four minor operations aged 2-9 years (grommets/circumcision).

The other 6 children were born at term and had surgery between one to four weeks afterwards. A further 2 had additional operations aged 1 year for atrial/ventricular septal defects and one had surgery at 9 years for a subaortic stenosis. These further operations had different skin incisions.

We were not able to get exact information about length of hospital stays in most children, due to the fact that they often stayed only for the time period of the operation at Great Ormond Street Hospital and were then in some cases discharged, but in others went back to the referring hospital, also the condition of the medical notes proved to be a problem. On average according to information from the Cardiac Intensive Care Unit, patients with an uncomplicated repair of aortic coarctation spent 10 years ago 1 day in intensive care, 7 days on the high dependency unit and a couple of days on a ward before discharge.

We were able to match participant factors possibly influencing our results as follows:
Participants were 9-12 years old at the time of testing. Both, scar and control group

had a mean age of 10.5 (± 0.8). The scar group and the control group included 4 girls and 5 boys. 8 children of the scar group were of Caucasian origin and one had parents that were originally Afro-Caribbean. In the control group 7 were of Caucasian origin and two had parents originally Afro-Caribbean. Most of the children and their parents had no exact knowledge of their height and weight. In the control group all children were of average build. In the scar group one child was obese. The number of right and left handed participants was the same in each group. We did not measure baseline skin temperature and also did not measure anxiety or fear at the actual testing appointment.

3.3.3 Inspection of scars

All scars were lateral thoracotomy scars in the region of the third to fifth intercostal space. 6 originated from a coarctation of aorta repair and 3 from a patent ductus arteriosus repair. The size varied between 12-24 cm in length (mean 18.85 cm) and 2-5 mm in width (variable within each scar). We took photographs of 8 scars; one child was not happy having a picture taken. Figures 3.7a-c show examples of the scars.

3.3.4 Questionnaire

Questions concerning the thoracotomy scars

One participant in the study reported that her scar felt slightly thicker than the surrounding area and less ticklish, she also stated that clothes, especially a bikini or crop top could feel a little uncomfortable. She noted the same

phenomenon with warm and hot water. The scars of all the other children were unremarkable.

Questions concerning pain experience in general

None of the children or their parents thought that they experienced more pain than their friends. Three of the children with scar (33.3%) and one from the control group (11.1%) reported that they experience less pain than their peers and their parents were in agreement with this.

One boy from the control group suffered from occasional chest pains that interfered with his life. Two of the children with scar who reported that they experienced less pain than their friends had pains that interfered with their lives (migraine (8/10), leg cramps (6/10)). The same two children had also high ratings concerning their most painful experience ever (8, 10). The average rating for this question in the scar group was 6.87 (± 2.69), in the control group 4.07 (± 2.62). The difference between the groups was not significant ($p=0.071$).

As in the previous group we compared the numbers of different pains experienced between scar group (SG) and control group (CG) (SG: mean = 3.22, $SD \pm 0.97$; CG: mean = 3.44, $SD \pm 0.76$; $p = 0.510$), amount of worst intensity pain from these pains reported (SG: mean = 7.44, $SD \pm 2.30$; CG: mean = 6.28, $SD \pm 2.34$; $p = 0.3707$). There were no statistically significant findings.

As a final analysis of general pain experience, the results of the amount of worst intensity pain were compared with the results of the thresholds from the respective reference area. This did not reveal any common trend.

3.3.5 Sensory testing

Overall, the testing procedure was well tolerated. No child withdrew from the protocol and no one reported discomfort or distress.

3.3.5.1 *General comments concerning effects of variables on threshold*

Initial analyses (nested ANOVAs) revealed that, for von Frey perception and cool perception in all participants, there was a significant effect of participant group (von Frey perception, $p = 0.000$, $F = 20.10$, $df = 1$; cool perception, $p = 0.015$, $F = 6.59$, $df = 1$), and site (von Frey perception, $p = 0.000$, $F = 35.88$, $df = 2$; cool perception, $p = 0.015$, $F = 4.80$, $df = 2$) on threshold. Furthermore, an interaction was noted between group and site that significantly affected thresholds (von Frey perception, $p = 0.015$, $F = 4.76$, $df = 2$; cool perception, $p = 0.048$, $F = 3.35$, $df = 2$). A similar pattern was found for warm perception, the only difference between this and the other 2 modalities being the lack of a group effect in this analysis, although the effect of site was significant ($p = 0.000$, $F = 33.32$, $df = 2$), as was the interaction between group and site ($p = 0.002$, $F = 7.30$, $df = 2$). For all modalities, there was no individual participant effect across the 2 groups (von Frey perception, $p = 0.283$; cool perception, $p = 0.09$; warm perception, $p = 0.072$).

3.3.5.2 *Scar Area (Table 3.1)*

Touch perception with von Frey hairs

When using von Frey hairs at the site of the neonatal scar, we tested on the scar (area 1) and next to the scar (area 2) (Figure 3.6). For the group comparisons though, we used only the results obtained on the scar, as there was no difference between

these two areas: Area 1: mean = 9.82, SD \pm 1.97, Area 2: mean = 8.67, SD \pm 1.14, p = 0.4505. Using only one result from the scar site gave equal numbers of test sites in the scar and control group and allowed for a balanced design in the statistical analysis.

Following the initial ANOVA (see Section 3.3.5.1), Tukey's post hoc comparisons showed in the participants with neonatal scar significantly decreased sensitivity to touch over the neonatal scar site (mean=9.82, SD \pm 1.97) compared to the contralateral control site (mean=6.82, SD \pm 1.88, p=0.0032), and compared to the reference area (mean=5.02, SD \pm 1.59, p=0.0000). The scar site also was less sensitive compared to the left thoracic (mean=6.55, SD \pm 1.37, p = 0.0012) and the right thoracic site (mean=6.67, SD \pm 1.37, p = 0.0019) of the control participants. Results are shown in Figure 3.8.

Cool perception

Once again, following the initial nested ANOVA (see Section 3.3.5.1), Tukey's post hoc comparisons revealed in the participants with neonatal scar a significantly increased cool perception threshold or a decreased sensitivity over the neonatal scar site (mean=25.05°C, SD \pm 6.28) compared to the contralateral control site (mean=29.44°C, SD \pm 0.82, p=0.0140), and compared to the reference area (mean=29.26°C, SD \pm 1.84, p=0.0203). The scar site also was less sensitive compared to the left thoracic (mean=29.49°C, SD \pm 2.20, p = 0.0127) and the right thoracic site (mean=29.84°C, SD \pm 1.12, p = 0.0061) in the control group. Results are shown in Figure 3.9.

Warm perception

Testing this modality revealed the same pattern: Tukey's post hoc comparisons showed in the participants with neonatal scar a significantly increased warm

perception threshold or decreased sensitivity over the neonatal scar site (mean=41.46°C, SD±3.71) compared to the contralateral control site (mean=37.08°C, SD±2.00, $p = 0.0004$), and compared to the reference area (mean=34.84°C, SD±0.87, $p = 0.0000$). The scar site also was less sensitive compared to the left thoracic (mean=29.49°C, SD±2.20, $p = 0.0127$) and the right thoracic site (mean=29.84°C, SD±1.12, $p = 0.0061$) in the control group. Results are shown in Figure 3.10.

Abnormal warm perception

A further finding was a difference in the way the sensation of warm was perceived on the scar site compared to the contralateral control site by the children who had an operation as a neonate. 8 out of 9 participants reported that they did not feel warm as such but immediately a hot sensation. Additionally 3 reported to have had a very brief feeling of cool before the hot sensation. The child who did not report this “hot” sensation was the obese one. This finding is illustrated in a diagram in Figure 3.11.

Brush allodynia

Two of the children with neonatal scar felt slightly uncomfortable when stroked with the brush over the scar (mean rating: 2.63, SD ± 6.63) and the same children reported slight discomfort over the contralateral control site (mean rating: 4.11, SD ± 10.48). None of the children from the control group reported this phenomenon.

Summary

The neonatal scar site showed a significantly decreased sensitivity in all tested modalities compared to all other sites tested.

3.3.5.3 Thoracic Control Areas (Table 3.1)

Touch perception with von Frey hairs

Sensitivity to touch perception of the left thoracic (mean=6.55, SD±1.37) and right thoracic sites (mean=6.67, SD±1.37) of the control participants did not differ from each other ($p=1.0000$) nor did the control site (right thoracic) (mean=6.82 SD±1.88) of participants in the scar group ($p=0.9991$ and 1.0000 respectively).

Cool perception

Once again, sensitivity of the left thoracic (mean=29.49°C, SD±2.20) and right thoracic sites (mean=29.84°C, SD±1.12) of the control participants did not differ from each other ($p=0.997$) nor did the control site (right thoracic) (mean=29.44°C, SD±0.82) of participants in the scar group ($p=1.0000$ and 0.9995 respectively).

Warm perception

For warm perception as well, sensitivity of the left thoracic (mean=39.22°C, SD±2.35) and right thoracic sites (mean=38.71°C, SD±1.72) of the control participants did not differ from each other ($p=0.9942$) nor did the control site (right thoracic) (mean=37.08°C, SD±2.00) of participants in the scar group ($p=0.8033$ and 0.4818 respectively).

3.3.5.4 Reference Area (Table 3.2)

Touch perception with von Frey Hairs

In the group of children (scar group) who had a lateral thoracotomy as a neonate the mean threshold was 5.02, SD ± 1.59 (results stated as von Frey hair numbers), whereas in the control group the mean threshold was 2.76, SD ± 0.786. This makes

the scar group significantly less sensitive to touch at the thenar eminence than the control group ($p = 0.043$).

The scar group reference area is also less sensitive to the scar area ($p=0.00$), but not compared to the contralateral thoracic site control site nor to the thoracic sites in the control group. The threshold of the control group reference area however is significantly less sensitive compared to any other area tested (scar group thorax right: $p=0.0001$, scar group thorax left (scar): $p=0.0000$, control group thorax right: $p=0.0001$, control group thorax left: $p=0.0002$). Results are shown in Figure 3.12.

Cool perception

There was no significant difference in threshold between the two groups in the reference area. (Scar group: mean= 29.26°C , $\text{SD}\pm 1.84$, control group: mean= 29.92°C , $\text{SD}\pm 1.30$).

There was also no difference of either reference area to the contralateral control site in the scar group, or to both thoracic sites in the control group, but both groups showed at their reference areas increased sensitivity for cool perception compared with the scar site (Scar group: $p=0.0203$, control group: $p=0.0051$). Results are shown in Figure 3.13.

Warm perception

There was again no significant difference in threshold between the two groups in the reference area. (Scar group: mean= 34.84°C , $\text{SD}\pm 0.87$, control group: mean= 34.50°C , $\text{SD} \pm 1.06$).

However, both reference areas were more sensitive for warming compared with the neonatal scar site (scar group: $p=0.0000$, control group: $p=0.0000$), the left thoracic site of the control group (scar group: $p=0.0087$, control group: $p=0.0032$), and the right thoracic site of the control group (scar group: $p=0.0021$, control group: $p=0.0007$). There was no difference between the reference area thresholds in both groups and the right thoracic site of the scar group. Results are shown in Figure 3.14.

Brush allodynia

Two of the children with neonatal scars felt slightly uncomfortable when stroked with the brush on the reference area. Their mean rating (out of 1-100) was 1.93, SD \pm 4.11. None of the control children reported this phenomenon.

The results from all individual participants in both groups were compared with the overall group comparisons and no discrepancies were found.

3.4 DISCUSSION

3.4.1 Study purpose and design

The aim of this project was to investigate any long-term consequences of neonatal injury upon somatosensory processing. We wanted to look especially at consequences that could be measured in an objective, reliable and repeatable manner. The idea was to investigate overall changes in sensory perception as well as specific changes of the injured and therefore scarred area of the body. Furthermore we were interested to see if there were any reports of unusual sensations or reaction around the injured area.

For our experiment we used a group of participants who had been injured as neonates (scar group) and a control group who had had no injury. Following our experience described in Chapter 2 we decided to find a group with an injury that had left a large, visible scar to facilitate testing and one that was on the same unilateral area of the body in each participant to allow for comparisons. We found this type of scar in children that had undergone a lateral thoracotomy in infancy.

We started our experiment with a questionnaire in which we asked general questions about health and demographics to make sure we met our in-/exclusion criteria and had two groups that were matched with regards to factors which could influence our testing results (see below). We further asked questions about the thoracic scar and general pain experiences.

We then used quantitative sensory testing as an objective measure of perception, measuring touch perception with von Frey hairs as well as warm and cool perception with a thermotest.

As a reference area and to compare overall sensitivity changes we chose the thenar eminence as this area has been used in the literature before (Hilz et al., 1996, 1998a,b; Meier et al., 2001) and there are reference intervals for thermal testing available (Hilz et al., 1998a).

We then tested the scar area and as control areas the contralateral site in the same participant and the same two sites in the participants of the control group.

3.4.2 Study findings

Our significant findings were as follows:

1. The neonatal scar area showed significantly decreased sensitivity to touch, cool and warm perception compared to all other sites tested in both scar and control groups. Thresholds for all modalities were significantly lower on the contralateral side in the scar group, and both thoracic sites in the control group. Indeed, there were no significant differences in threshold between any of these three sites.
2. On testing for warm perception on their scar the neonatal scar group reported in 8 out of 9 cases that there was no warm sensation but that the area immediately felt hot. Three of the participants had a brief cool sensation beforehand.

3. The reference area showed significantly decreased sensitivity to touch in the neonatal scar group compared to the control group. This was not the case with cool and warm perception.
4. Two of the children with scar reported that brushing the skin on any site tested was mildly unpleasant.
5. One out of nine children in the scar group reported in the pain questionnaire signs of allodynia over her scar.

3.4.3 Interpretation of results

Our results show a decreased sensitivity to touch at the thenar eminence ten years after the children had an injury/operation as a neonate. This means a significant injury early in life can cause generalized changes in tactile sensory processing. The same change in sensitivity was not demonstrated for cool and warm perception nor was it shown at thoracic level. It is not possible to conclude from these results if pain processing is altered or not.

3.4.3.1 *Mechanisms Underlying Baseline Hyposensitivity*

We discovered hyposensitivity at a higher spinal segmental level (reference area equals C6) than the original injury had been (T4). This suggests that although the mechanisms causing this general hyposensitivity were triggered by the original insult, they extend far beyond the segmental sensory and nociceptive circuitry belonging to

the site of the neonatal injury, and are rather more global, involving non-somatotopic factors. There are animal studies that also support a more generalized long-lasting hypoalgesia following early injury. Traub and co-workers, and Wang and co-workers (both 2003) found an exaggerated visceral hypoalgesia in animals which experienced carrageenan (CAR)-induced inflammation of a hindpaw shortly after birth. Ren (2004) observed that adult animals which experienced early inflammatory insult suffered from long-term hypoalgesia in the affected hindpaw, the contralateral paw and both forepaws. We suggest that these global effects involve the hypothalamic-pituitary-adrenocortical axis as described by Tsigos and Chrousos (2002), or perhaps alterations in the corticolimbo-brainstem descending pain/stress modulatory circuitry (Fields and Basbaum, 1999; Keay and Brandler, 2001). A trigger for long-term changes of baseline sensory perception could be the stress these children encountered during their surgery and hospital stay as infants.

A number of neurodevelopmental and behavioural changes in very low birth weight children, such as deficits in cognition, learning disorders, poor motor performance, behaviour problems and attention deficits with academic problems persisting into adulthood are reported. These long-term sequelae seem to be attributable to early pain and stress in NICU (Grunau, 2002). Other authors (Jacobson et al., 1987, 1998) related adult self-destructive behaviour with traumatic experiences around the birth of these individuals and suggested that imprinting at birth may predispose individuals to certain patterns of behaviour that remain masked most of the time but may come out during conditions of extreme stress. Anand and Scalzo (2000) propose two hypotheses to link perinatal sensory experiences and adult behaviour: (i) excessive neonatal stimulation resulting from perinatal trauma or other insults causes NMDA-mediated excitotoxicity in multiple areas of the developing brain, and (ii) lack of appropriate sensory stimulation (e.g. maternal separation) in the neonatal period

serves to enhance the normal occurrence of developmental apoptosis in the neonatal brain.

In animal models neonatal stress is most often mimicked by handling and maternal separation. This type of stress can have various outcomes which again seem closely related to the type of stress, but also to maternal care-giving practices. Daily handling for 14 postnatal days increased nociceptive thresholds in adult rats and decreased behavioural responses to stress (Sternberg and Ridgeway, 2003). D'Amore (1995) showed the same effect for noxious stimulation in adult mice. This type of "slight stress" caused in adult rats in stressful situations a cortisone release which was lower and of shorter duration than in controls (Meany et al., 1988), whereas "severe stress" led to visceral hyperalgesia and reduced somatic analgesia (Coutinho et al., 2002) and to an increased sensitivity to stress (Plotsky and Meany, 1993) in adult rats. Further studies supported the observation that the mother's tactile contact with the pups influenced their future reaction to stress (see Winberg, 1998), the more "tactile" mothers' offspring showed a lower corticosteroid release during stress. Relating these models back to the group of children we examined one would then assume that their experiences as neonates were "slight stress" and that they were looked after well by their mothers/caregivers.

In our study we found decreased baseline sensitivity for touch perception but not for cool and warm perception between the scar group and the control group. There are a number of reasons why this may have occurred:

Firstly touch and thermal perception are different sensory modalities and transmitted by different fibres. A α and β fibres convey light touch perception, A δ non-nociceptive fibres cool perception, and unmyelinated C fibres warm as well as noxious perception (Vinik et al., 1995, Verdugo et Ochoa, 1992). These fibres could react differently to the inflicted injury leading to different consequences for sensory transduction and

transmission. A global effect such as decreased baseline sensitivity to touch is however, as discussed previously, much more likely to originate in a central mechanism. This would imply differential central processing of touch and thermal perception, but since central pathways are mixed (Ganong, 1997), this is an unlikely explanation for our finding.

Another reason for the difference in baseline touch but not in thermal perception could be that a difference in thermal perception between the groups was present but we were unable to measure it because of our technique. For testing touch perception we used a much smaller and more punctate stimulus. Von Frey hairs range in their diameter from 0.08 mm to 1 mm (Andrews, 1993). Our thermode for thermal testing was in comparison 18 x 18 mm in size. For this reason it may have been possible to detect much more subtle changes in mechanical sensation than in thermal sensation. A further reason could be that our participants were more sensitive to the change of touch from 17.4mg to 29.20 mg etc. (see von Frey Hair table, Figure 2.1), than to a temperature change of 1°C/s.

A further point is that we detected a baseline sensitivity change in touch perception between our cardiac surgery group and the control group at the reference area (thenar eminence), but not at the thoracic control site – the contralateral site of the initial injury. One possible reason is that our equipment could not detect small changes in the thoracic region. The thoracic region of the body is less sensitive to touch stimulation than the hand due to a lower receptor density (Kandel et al., 2000). In our own control group the touch perception threshold at the thenar eminence was a mean of 2.76 (von Frey hair number) and at the thoracic sites a mean of 6.55 and 6.67 respectively. The weight applied by hair number 2 is 29.2 mg, 3 is 47.9mg, and those applied by hair numbers 6 and 7 are 219mg and 363 mg respectively. Bell-

Krotoski and co-workers (1995) found 2 filaments from different manufacturers one applying a mean weight of 62mg and one applying a mean weight of 95mg both to be good predictors of normal touch sensation of hands and arms.

Our control group showed a difference for touch perception and for warm perception between reference area (C6) and thoracic areas (T4), but not for cool perception. Some of our control participants mentioned that they found it more difficult to determine the feeling of warm than that of cool specifically at the thoracic site. This is reflected in a greater standard deviation of the warm sensation results compared to the cool sensation results. There is no comparison of these segmental levels in the literature, perhaps because the main clinical interest lies in peripheral neuropathies or nerve injuries of limbs and face.

Our results show that the neonatal scar area has a significantly decreased sensitivity to touch, cool and warm perception compared to all other sites tested. There was no difference in threshold in any modality in between the contralateral site in the scar group and the thoracic sites in the control group. An important point to note is that there is a significant difference between the injured site and the contralateral control site on the same segmental level in the scar group, not only compared to the control group. This seems to indicate that the mechanisms causing these very distinct threshold differences in all modalities are mainly due to changes caused at the actual site of wounding or in the somatosensory circuitry involving specifically the injured side at its segmental level.

The observation that two of the participants with scar felt that brushing of their skin at all tested sites was mildly unpleasant suggest that there may be other more complex baseline sensory effects. However this altered perception was a weak effect and it is hard to draw firm conclusions from it.

3.4.3.2 *Mechanisms Underlying Decreased Sensitivity at the Scar*

One possibility would be generalized damage to all sensory afferent nerve fibres in the scarred tissue, which would cause long term hyposensitivity over the injured area. In human adults, touch and temperature thresholds are each altered by superficial skin wounds, but 4 weeks after injury they have returned to pre injury levels (Hermanson et al., 1987). Zhang and Laato (2001) examined samples of normal skin, normal and hypertrophic scars, showing in the normal scar tissue fewer nerve fasciculi compared to normal skin. This could explain an increased perception threshold. These scars though were not due to neonatal injury. We also found hyposensitivity 3-5 mm next to the scar when testing with von Frey hairs, which points to a wider involvement than only the actual scar tissue.

In animal models following skin wounding at birth, wounds heal rapidly, but the sensory nerve terminals in the area show a profound sprouting response, which long outlasts the injury (at least 12 weeks in the rat) (Reynolds and Fitzgerald, 1995, De Lima et al., 1999, Alvares et al., 2000). This effect decreases progressively with age at wounding and is a sensory A and C fibre nerve response with no sympathetic involvement (Reynolds and Fitzgerald, 1995). Behavioural studies show that the hyperinnervation is accompanied by long lasting hypersensitivity and lowered mechanical thresholds in the injured area (Reynolds and Fitzgerald, 1995, De Lima et al., 1999). In addition, this wounding leads to a long-lasting expansion of dorsal horn cell fields (Torsney and Fitzgerald, 2003). The hyposensitivity we found in the participants of our study seems to be contrary to these observations of hypersensitivity. All these findings implicate changes in the segmental circuitry of the wounded area that seem to increase the sensitivity to future perception and damage. But as demonstrated for the central auditory nervous system and in other neural systems (Vale & Sanes, 2002; Chapman & McKinnon, 2000; Kolb et al., 2000) a

neonatal insult is not only capable of facilitating excitatory changes but also of increasing inhibitory mechanisms. There is the possibility of initially enhanced sensory input from "hypersensitive" skin leading to adaptive changes within the spinal cord dorsal horn that decrease the receptive field size of afferent neurons (Rahman et al., 1997). The reduction in receptive field size may be caused by reduced dendritic arborisation of afferent neurons, increased connections with inhibitory interneurons, up-regulation of inhibitory receptors or ion channels, or enhanced activity of the descending inhibitory controls from supraspinal centres (Ren et al., 1997).

3.4.3.3 *Aberrant Warm Sensations*

An extremely interesting phenomenon occurred when testing the scar for warm sensation: 8 out of 9 participants from our scar group had no warm sensation like they had felt at the reference area, but felt immediately hot, some painfully so. In 3 participants, this was preceded by a momentary feeling of cool or cold.

Warm sensation is conveyed at the primary sensory neuron level by unmyelinated C fibres and has specific warm receptors that are optimally excited in the innocuous temperature range. Unmyelinated C fibres also mediate heat pain sensation, but the receptors are polymodal C nociceptors (Hallin et al., 1982). Additionally there is a minor A δ contribution to heat pain for hairy skin. In general heat pain evoked during quantitative sensory thermotest stimulation has a burning quality, as opposed to a sharp prickling quality. The former corresponds to excitation of C- polymodal nociceptors while the latter corresponds to excitation of A δ nociceptors in humans (Verdugo and Ochoa, 1992).

In adult small fibre neuropathies, of whatever cause, the drop out of C unmyelinated fibres is expected to blunt warm sensation, while sparing thermal pains. This occurs

because, as opposed to C mediated warm sensation, C mediated thermal pains have little requirement for spatial summation, i.e. the threshold function remains normal for thermal pains despite a marked depopulation of afferent fibres. (Verdugo and Ochoa, 1992). A possible depopulation or destruction of fibres in the scar tissue could therefore explain our observation. Unfortunately there is no reference data in the literature about normal thermal pain thresholds in the thoracic area for comparison with our result.

A more complex explanation however could be that injury at the critical stage of postnatal organization and maturation in the dorsal horn of the spinal cord of the central terminals of primary sensory afferent neurons (Fitzgerald, 1995) could cause an inhibition of the normally occurring A-fibre withdrawal due to possible destruction of afferent C fibres (Shortland et al., 1990). Unfortunately we did not ask the participants of our study about the quality of the hot sensation they perceived, therefore we have no indication if this sensation was A- or C-fibre mediated.

Only one of the participants reported, when asked in the questionnaire, findings consistent with very minor allodynia over the scar (discomfort when wearing crop top), none of the others felt any difference, we specifically asked for warmth (e.g. water). Therefore it seems that the sensory changes found whilst testing have no importance for most of the children. The reason could be that the scars are relatively narrow (2-5mm) and therefore an odd sensation just on the scar is not really noticed; or it might be the location: one would be probably much more aware of a similar scar at the hand with these sensory deficits than of one at the lateral thorax.

3.4.3.4 Summary of Mechanisms

Amassed evidence points towards one important mechanism for long-term consequences of neonatal injury and stress – activity dependent plasticity of the pain pathways (Fitzgerald and Walker, 2003), with all their anatomical, physiological and neurochemical characteristics. These pathways are shaped during a critical period of development by learning from the altered input noxious and stressful stimuli provide.

On the physiological side there is permanent hyperinnervation of the wound site (Reynolds and Fitzgerald, 1995), long-lasting changes in primary afferent projections to the dorsal horn (Ruda et al., 2000), increased receptive field size of adult dorsal horn neurons (Torsney and Fitzgerald, 2003) or change of normal development of spinal sensory connections by blockade of NMDA activation (Beggs et al., 2002). On the behavioural side there are animal reports about changes of adult pain and stress behaviour (e.g. Ren et al., 2004, Sternberg and Ridgeway, 2003) and it is well known that even very premature neonates actively perceive, learn, and organize information, while constantly striving to control their sensory input (see Anand and Scalzo, 2000).

3.4.3.5 Comparison with QST Literature

QST has been widely used in adults to examine various medical conditions involving peripheral neuropathies, diseases of the CNS or trauma in the adult (Zaslansky and Yarnitsky, 1998). There are normal reference values available for vibration and thermal testing mainly for hands and feet (Bartlett et al., 1997; Yarnitsky and Sprecher, 1994). Normal sensory detection thresholds for the entire body, and the stimulus force for each monofilament, were determined by Weinstein (Bell-Krotoski et al., 1995).

After analyzing the QST results of 465 adult patients with somatosensory dysfunction, Verdugo and Ochoa found that thermal specific (warm or cold) hypoaesthesia and thermal hyperalgesia may occur in the absence of hypoaesthesia for tactile sub modalities served by large calibre afferents. They found 13 abnormal patterns of thermal hypo-and hyperaesthesias. This shows that almost any variety or combination of dysfunction and normality regarding the different modalities is possible.

QST in children has been used much less widely. Hilz and co-workers (1998; 1996) and Meier and co-workers (2001) investigated normative values for thermal and vibration sensation and thermal pain detection thresholds in children and adolescents. The thresholds for cool and warm sensation for the reference area of both control and scar groups in our study are within the normal reference intervals of the Method of Limits examinations at the thenar eminence reported by Meier and co-workers (2001) and Hilz and co-workers (1998). These two investigators also looked at influence of body side, skin temperature, size of probe and reproducibility of results.

In the clinical setting thermal and vibration testing has been used to assess children with diabetic neuropathy (Heimans et al., 1987; Abad et al., 2002) and pressure algometry has been used to assess pain thresholds in rheumatoid arthritis (Hogeweg et al., 1995).

One paper by Thibault and co-workers (1994) also describes normative values for touch perception in children, examined with Semmes-Weinstein monofilaments. The results of this group are similar to the results of the reference area of our control group at the same segmental level, whereas the results at the same level of our scar group are higher, which supports our result of generally decreased sensitivity to touch in our scar group.

The reason for very few QST reports involving children might be that it is a rather time consuming investigation that needs a lot of focus and concentration. These two aspects combined might prove a challenge in a lot of children, especially the very young. In our study we found that our timescale (about 35 min.) was just at the border of tolerance for our participants and concentration varied a great deal depending on the time of the day, whether testing was on Saturdays or after school, and also on the input of the attending parent.

There are very few papers using QST to assess pain/nerve function after wounding or surgery. In adults Wilder-Smith and co-workers (1999) used electrical stimulation to assess skin sensory thresholds up to 4 days postoperatively. Moinche and co-workers (1997) used pressure algometry up to 8 days after hysterectomy at the wound site and at a distant site. They found increased sensitivity around the wound, but not at a distant site. Stubhaug and co-workers (1997) and Richmond and co-workers (1993) used von Frey hairs to evaluate the effects of morphine and ketamine. They evaluated patients/wounds for 48 hours and 7 days respectively.

In neonates and infants under 1 year, assessment with von Frey hairs in connection with wounding has only been done by Andrews and co-workers (2002a and b). Von Frey hairs were applied preoperatively, immediately after surgery and in the clinical follow-up 3 months after surgery to quantify wound sensitivity in infants following abdominal surgery using the abdominal skin-reflex.

In contrast to our study all the ones mentioned above tested their participants only in a relatively short time-span after wounding/surgery; the longest interval being 3 months (Andrews et al., 2002b). Reasons for this could be that there is more interest in the immediate post-op clinical situation as health-care professionals are more involved with their patients during this time. At this time point recruitment for a study is also

much easier as the participants are readily available; asking for a separate time-consuming visit is much more difficult, especially where children and parents are concerned. Wounding, scarring, and pain are for the majority of affected individuals obvious and more problematic in the early stages, but at later stages often get neglected. Grunau and co-workers (1993; 1998) have followed pre-term newborns until the age of 10 and found that on the whole the children judged pain in pictures similarly to their term peers, but did no actual somatosensory testing. Therefore our study provides the unique opportunity of a follow-up up to 12 years after the initial insult. This is even more important as there is emerging evidence that permanent functional deficits may take a prolonged time to reach their final expression, especially if the effects are global.

All the QST studies cited regarding wounding concentrated their testing on local effects and at the most looked at general pain by using verbal reports or visual analogue scales, but not QST. Our study is the only one that also investigated generalized somatosensory changes.

A study by Malenfant and co-workers (1998) looking at tactile, thermal and pain sensibility in adult burned patients deserves to be mentioned separately as it is the study with the longest time interval after insult (at least 18 months), the authors tested the injured site of the body and the contralateral control site and used tactile, thermal and pain thresholds for their assessment. The study contained several interesting findings. Sensory thresholds were significantly higher in burned patients than in control participants; and thresholds for touch and cold perception were mostly affected and highest. Interestingly, when comparing the thresholds of the contralateral uninjured site of the burned patients to those of control subjects the difference between the two sites in touch perception was most striking. In our study we found an

increase in baseline sensitivity for touch but not for thermal perception. Unfortunately these authors did not test a reference site at a different segmental level.

In a study of 60 adolescents (12-18 years) born prematurely tenderness thresholds were assessed on 18 tender point sites around the body (points as suggested by the American College of Rheumatology for assessing non-articular tenderness in studies of widespread pain and fibromyalgia) and 4 control sites (forehead, forearm, lateral knee and 3rd metatarsal) and compared with 60 age-matched controls (Buskila et al., 2003). The prematurely born children had significantly more tenderpoints and lower tenderness thresholds in both tendersites and control sites, the children did not however report any increased pain or stiffness in their daily life. Tenderness over these sites was determined with a dolorimeter at the rate of 1 kg/sec up to a maximum of 9 kilograms over a 1.4 cm diameter plate. The participant was requested to say yes when the sensation changed from pressure to definite pain. While these results may appear to conflict with our findings, in fact they are examining a different sensory modality to those reported here. While we have examined cutaneous thresholds to touch and temperature applied to the skin surface, Buskila and colleagues (2003) have focused on deep connective tissue responses to pressure pain at highly defined points relevant to rheumatology. It is therefore not possible to make a direct comparison between the two studies.

3.4.3.6 Sample and Methods

Only 37% of suitable participants with scars who were contacted were interested in coming to the testing which could raise the question of whether our sample was representative or self-selected. One possibility is that the other children were not able to attend because they had other health problems. We addressed this possibility by

contacting the children's GP's first and also looking at their Great Ormond Street Hospital medical notes. After this we excluded 3 children because of medical problems from invitation and a further 6 because of difficult family situations. We had a relatively small number of participants ($n=9$) in each group. One of the main problems which became obvious when organising appointments was that due to the age group investigated we needed parents to attend with their children, and this made arrangements very complicated for them. Nevertheless the effort involved meant that all the participants were highly motivated, a very important factor for quantitative sensory testing (Siao and Cros, 2003). Also, we adhered very strictly to our exclusion criteria and did not test any child whose somatosensory function could have been influenced by anything but factors beyond our control: for example little sleep the night before testing or testing appointment occurring after a long day at school, which may impact on the participant's concentrating abilities. We therefore feel that our sample was representative.

As a reference area we chose the thenar eminence because this is an easy accessible site and normal values for comparison were available (Meier et al., 2001; Hilz et al., 1998; Thibault et al., 1994). Our choice of reference area could be a point of controversy as all children in our scar group needed arterial lines for monitoring which are often sited into the radial artery and could have caused nerve damage at this site. Searching through the children's medical notes we found only one child who had had a radial arterial line, three had brachial, two posterior tibial and one child a line in the umbilical artery. In two cases there was no information available. These two had the lowest touch perception thresholds and the highest were found in the children who had had a posterior tibial arterial line.

Our testing environment was not ideal as we did not have a soundproofed, air-conditioned room. The room temperature in our testing room varied between 28 and

32°C, which at this level according to Meier and co-workers (2001) might influence the results for thermal testing, but as the temperature was always rather high, this should not influence the overall comparisons between our two test groups. We tried to keep the test room always in the same condition and minimize distraction and noise, although due to lack of soundproofing, noise outside the room was variable, and depended on the time and day of testing.

We carefully checked the equipment before every session and used the same standardised instructions for every participant. The thresholds for cool and warm sensation for both groups were tested with the “method of limits” examination reported by Meier and co-workers (2001) and Hilz and co-workers (1996; 1998). We chose this algorithm as others are more complicated and time consuming and might further bias the cooperation of such young volunteers. We chose to examine only cool and warm thresholds as we expected fewer volunteers of this age group and also fewer parents to agree to participate if we tested pain thresholds. Heat pain may have given us further information about mechanisms and fibres involved (Verdugo and Ochoa, 1992), but following our experience with the neonatal intensive care survivors (see Chapter 2), we believed that cold pain thresholds would be too inconsistent to give further information. We used an 18x18 mm probe, as this was the best size to align with the sites we tested (Hilz et al., 1998). For testing the actual scar a very small punctate thermode, which would allow us to test only the scar and the area immediately next to it, as we did with von Frey hairs may have yielded additional information, but unfortunately we did not have such a probe available.

The von Frey hairs we used were kept constantly in the testing room in the same conditions to avoid effects of changing environment on their accuracy (Andrews, 1993). We strictly adhered to our algorithm and the filaments were applied as recommended by Bell-Krotoski (1990). We noted that this examination was more

difficult for the children than temperature perception regarding concentration and boredom, especially when we tested on the scar and next to the scar.

Concerning concentration, especially in this age group, boredom and length of investigation definitely seemed to be influential. We noticed that the children started moving around more towards the end of the testing. To reduce the influence of this factor especially on the testing of the right and left thoracic sites, we alternated the side that was first tested. Another factor regarding concentration was the presence of the parents. They were seated at the other side of the room so it was impossible for them to give their children any clues regarding the actual testing process, but they were able to observe their children and their reactions and behaviour. This resulted in the occasional parental intervention by telling the child to not move around or to concentrate. It is impossible to say what influence these comments might have had on our actual test results.

Almost all participants found testing for brush allodynia a very pleasant experience and enjoyed it, but did not always seem to understand the reason for doing it.

Before we used the questionnaire for participants, we tested it on children of the same age and background, to see if they would understand the questions and were able to answer them, nevertheless we might have missed some specific points or expressions to get exactly the information we wanted.

When asking the questions the parents were present in the room, which might have influenced the answers (Grunau et al., 1993, 1998). Some of the children also asked the parents for help when they were unsure about an answer, which means that we may have got the answer from the parents' and not the child's perspective. In hindsight it would have been better to ask the parents to wait outside while we asked

the questions. We did not consider this option in the first place due to a lack of waiting area.

3.5 Summary and Conclusion Chapter 3

We investigated children with neonatal thoracic scars 9-12 years after the initial injury. Our participants were less sensitive to touch perception over the thenar eminence compared to the control group and their scar areas were less sensitive for cool, warm and touch perception than any other site tested. They also reported an immediate feeling of “hot” and the missing of a “warm” sensation over their scars. Comparing their answers with those of the control group in our pain questionnaire, there is no evidence of different pain experience in daily life.

Our study is the first ever investigation in humans looking with quantitative methods at somatosensory changes over a neonatal scar or any scar after a period of a decade. There is also no other human study looking at baseline or general changes in perception of the scarred individuals.

We showed that there are definite long-term somatosensory changes over the scarred area and a different perception to touch in general in children with big neonatal scars.

Further information could be gained by testing a group of children who have received a similar scar at an older age. This would allow us to find out if there is any difference in perception of any modality between those with a neonatal and those with a later scar.

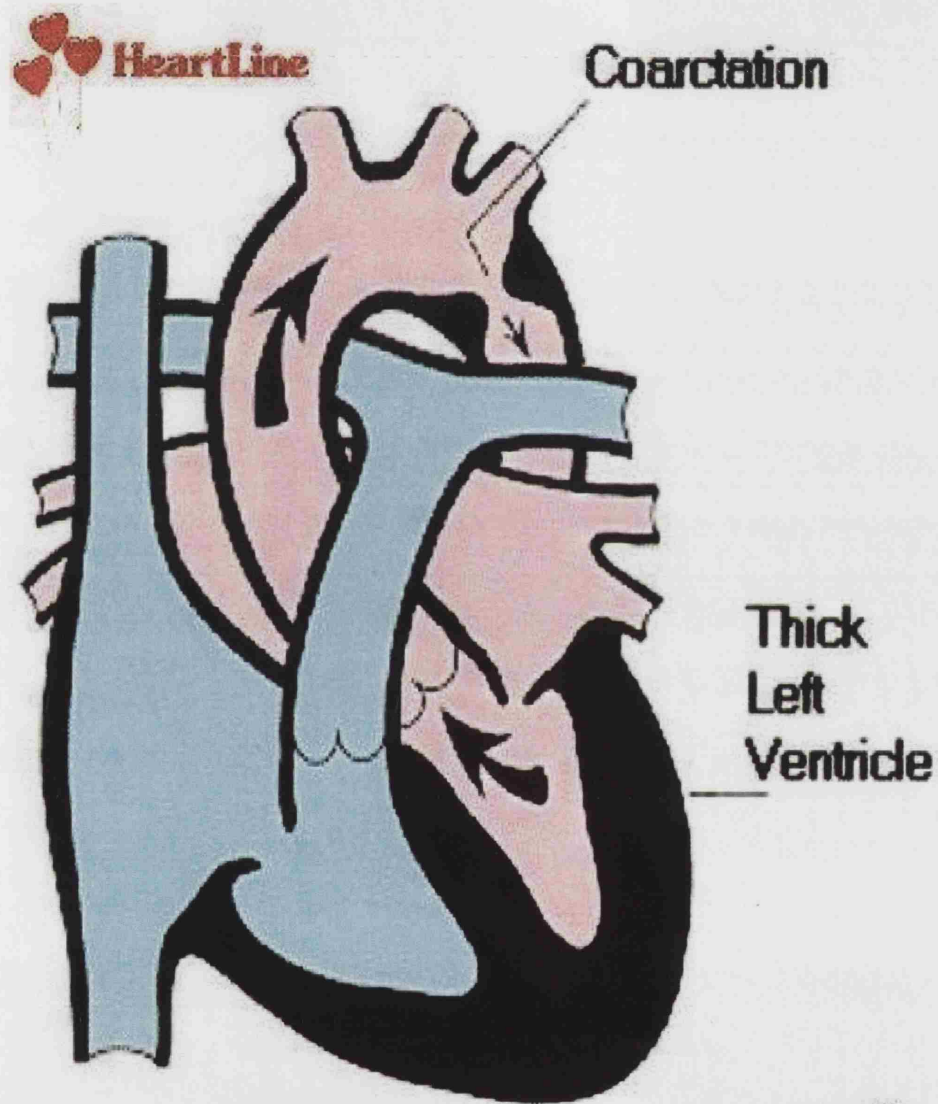
Table 3.1: Thresholds in the Thoracic Area

	Participants with neonatal scar (n=9)		Control participants (n=9)	
	Thorax left (scar)	Thorax right	Thorax left	Thorax right
Touch perception (von Frey hair number) Mean± SD	9.82 ± 1.97 (on scar) 8.67 ± 1.14 (next to scar)	6.82 ± 1.88	6.55 ± 1.37	6.67 ± 1.75
Cool sensation (°C) Mean± SD	25.05 ±6.28	29.44 ±0.82	29.49 ±2.20	29.84 ±1.12
Warm sensation (°C) Mean± SD	41.46 ±3.71	37.08°C ±2.00	38.22 ±2.35	38.71 ±1.72
Brush allodynia (rating) Mean± SD	2.63 ±6.63	4.11 ±10.48	0.00 ± 0.00	0.00 ± 0.00

Table 3.3: Thresholds in the Reference Area

	Participants with neonatal scar (n=9)	Control participants (n=9)
Touch perception (von Frey hair number) Mean \pm SD	5.02 \pm 1.59	2.76 \pm 0.78
Cool sensation (°C) Mean \pm SD	29.26 \pm 1.84	29.92 \pm 1.30
Warm sensation (°C) Mean \pm SD	34.84 \pm 0.87	34.50 \pm 1.06
Brush allodynia (rating) Mean \pm SD	1.93 \pm 4.11	0.00 \pm 0.00

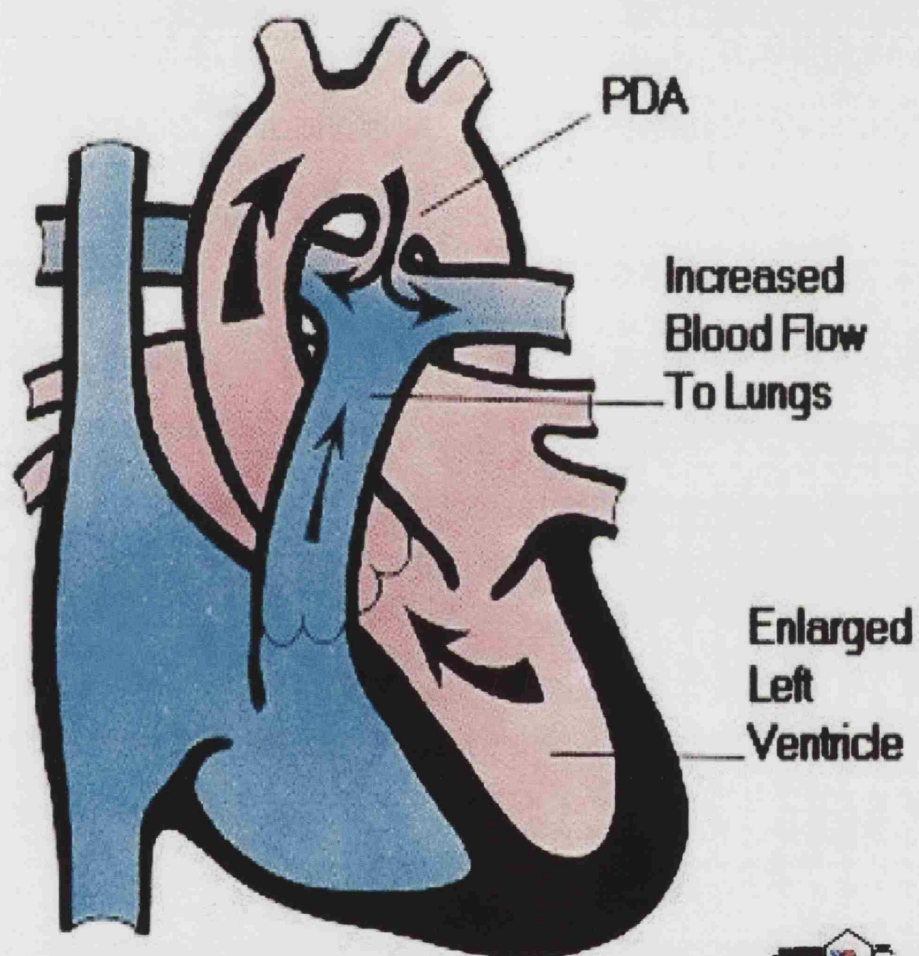
Figure 3.1:



COARCTATION OF AORTA

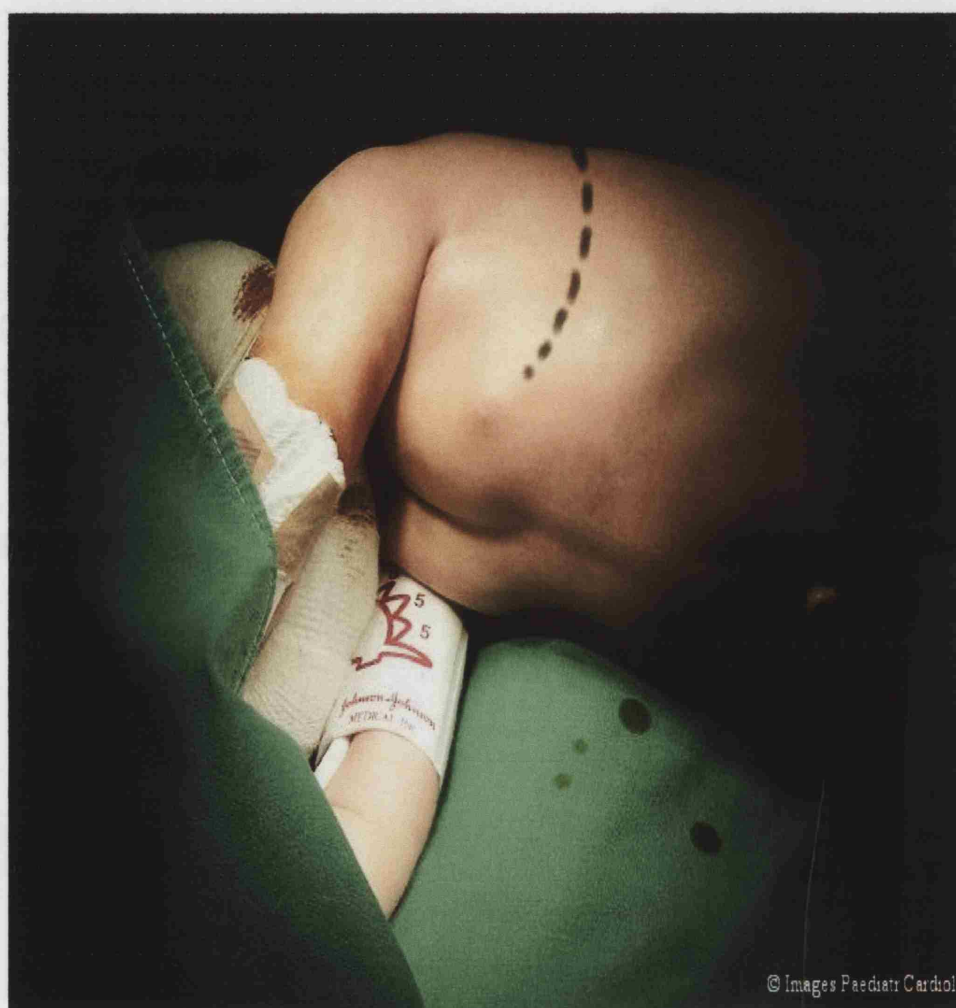


Figure 3.2:



PERSISTENT DUCTUS ARTERIOSUS

Figure 3.3:
Patient position (left lateral approach)



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Figure 3.4 :
A curved incision is made on the left thorax

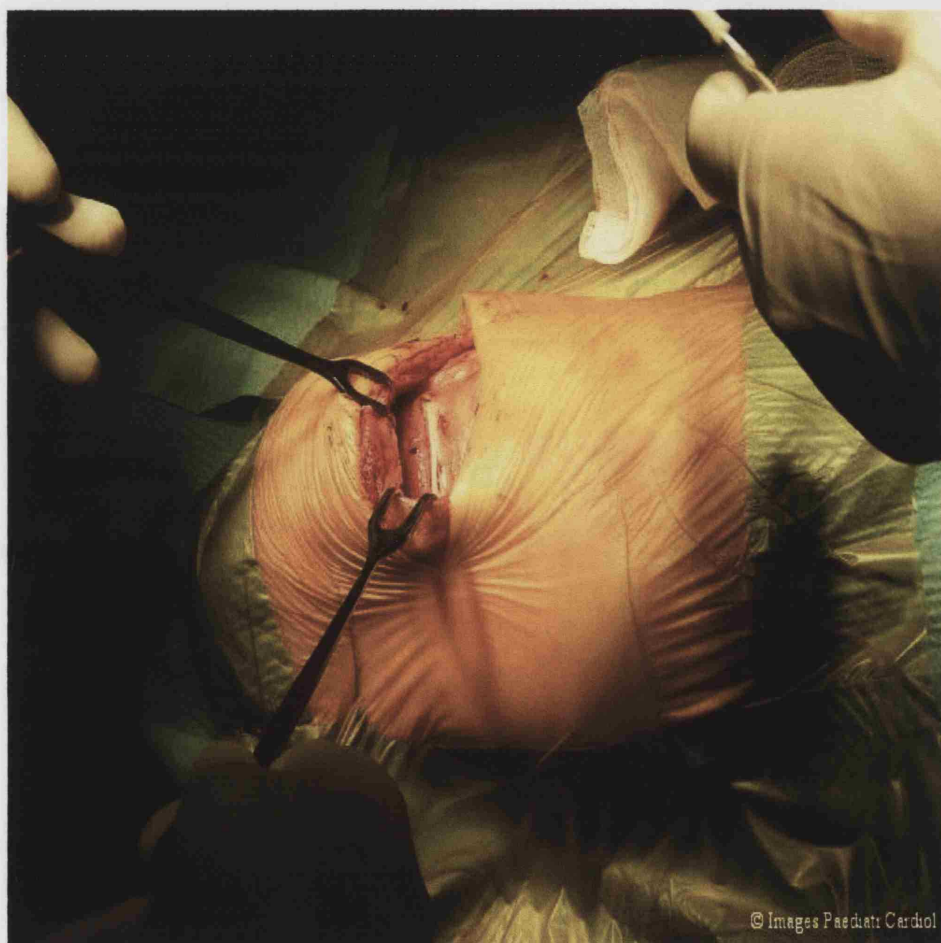


Figure 3.5 : Left lateral thoracotomy

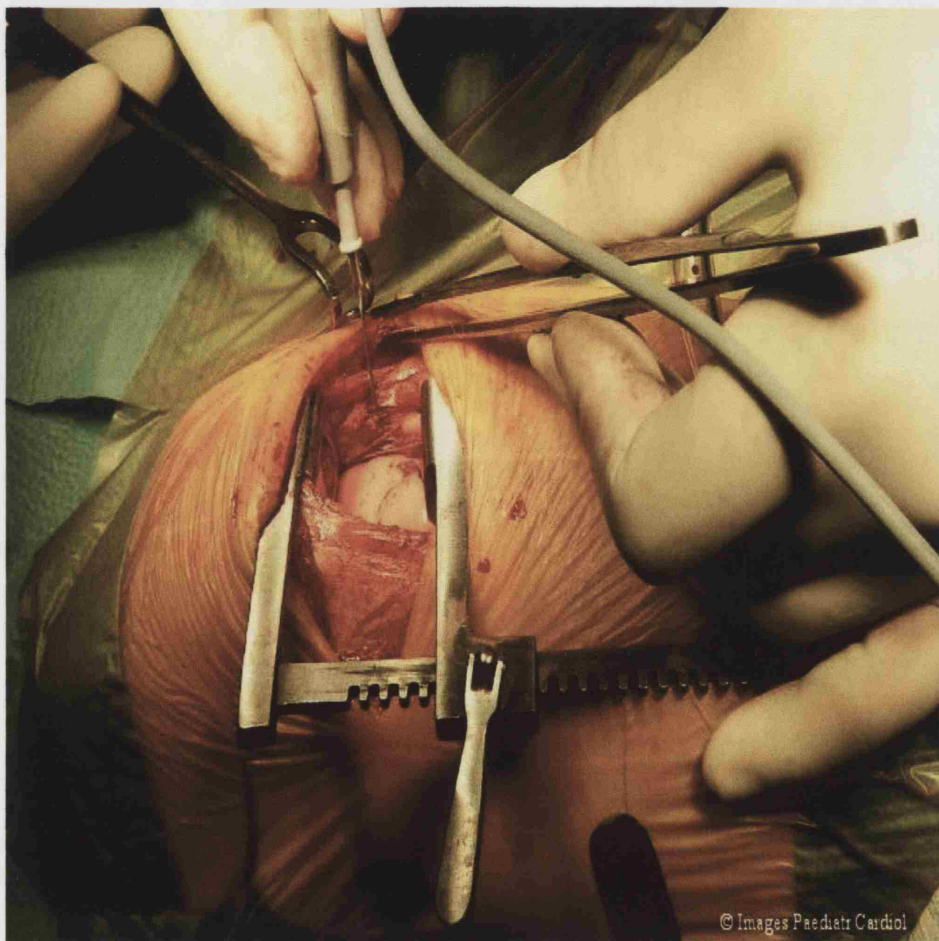


Figure 3.6: Skin areas tested

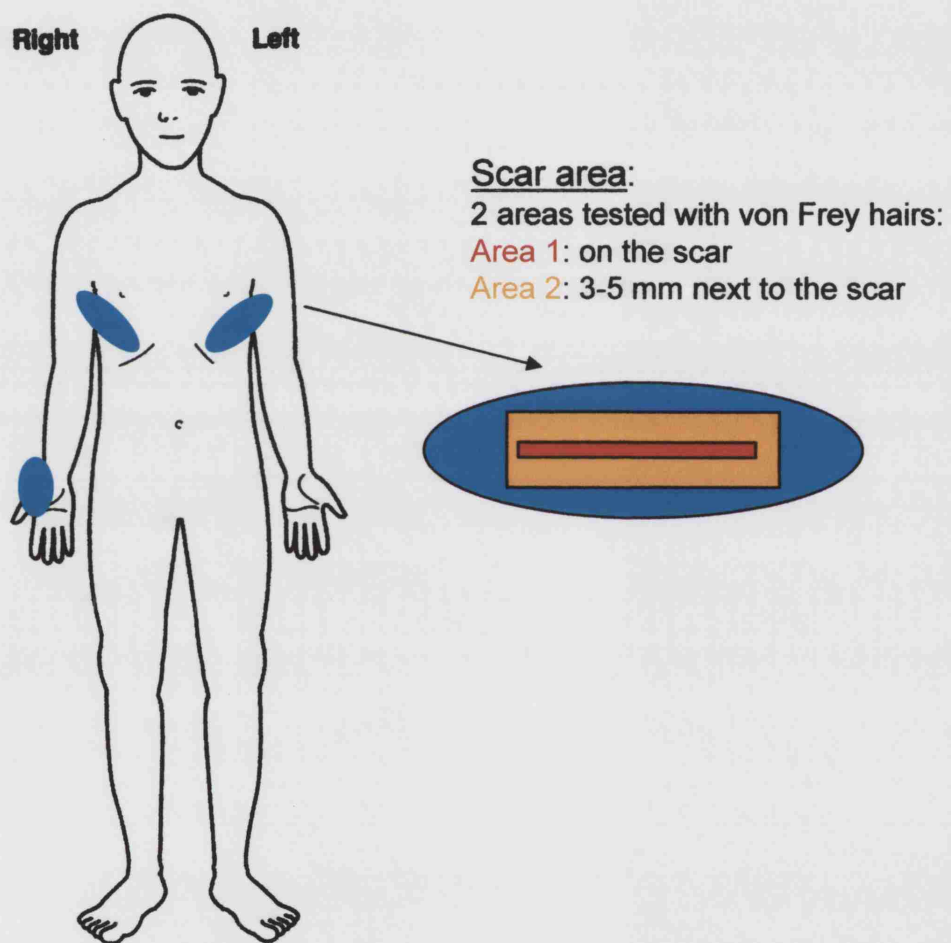


Figure 3.7a:
Example of Thoracotomy Scar

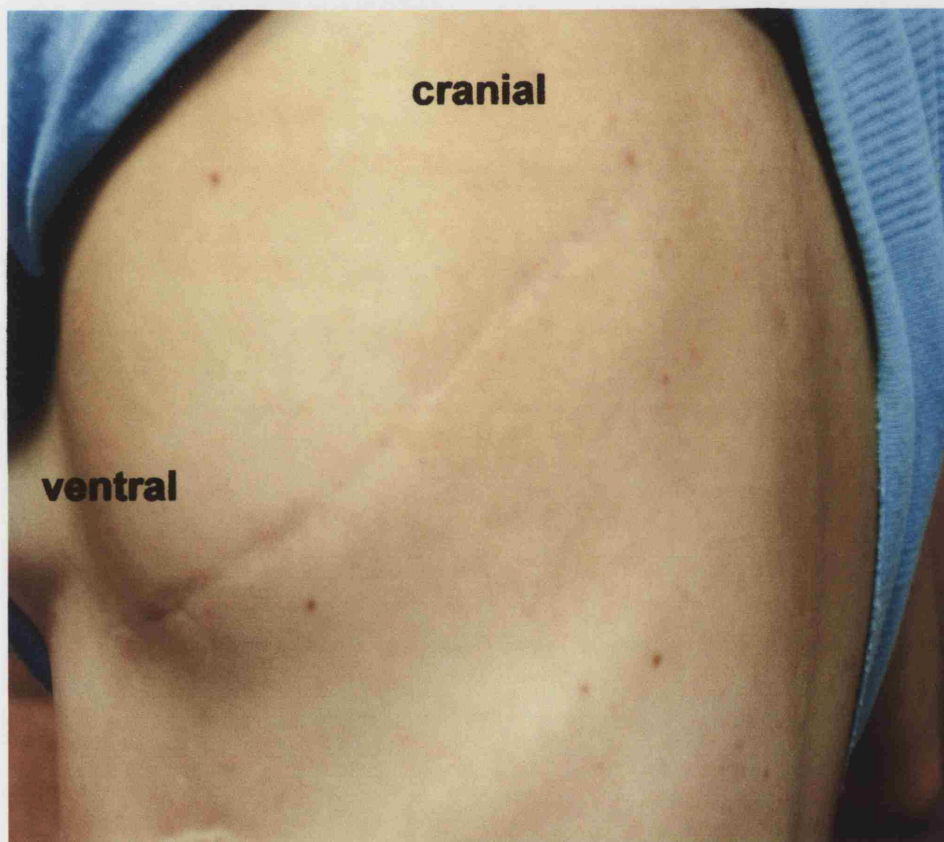


Figure 3.7 b:
Example of Thoracotomy Scar

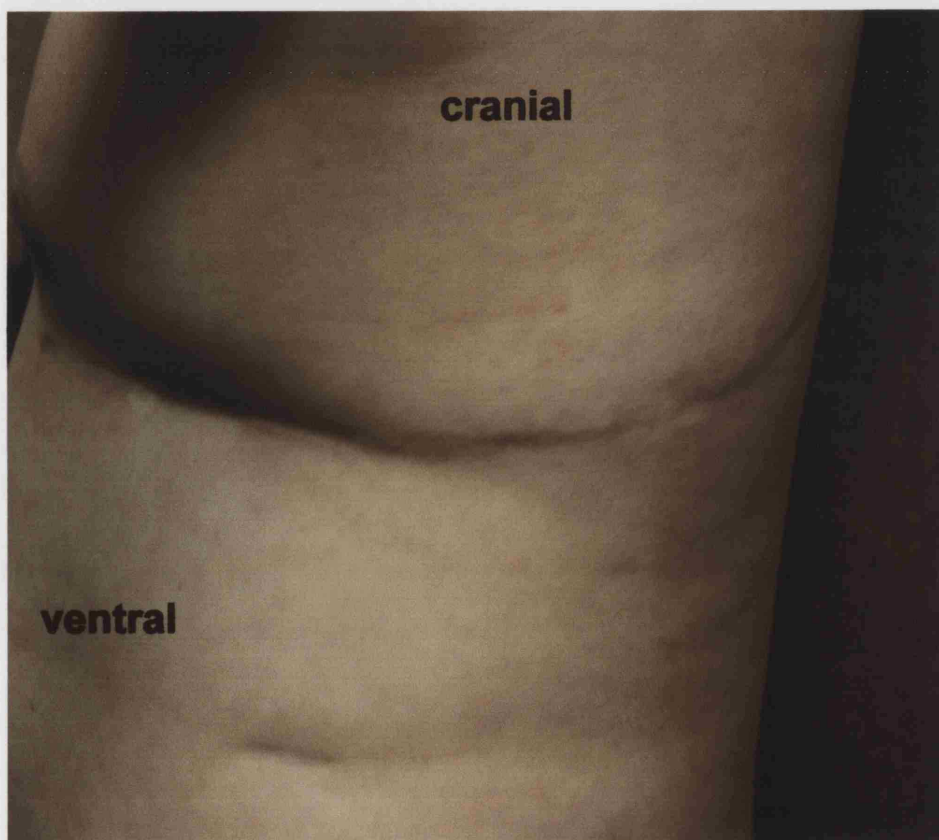


Figure 3.7c:
Example of Thoracotomy Scar

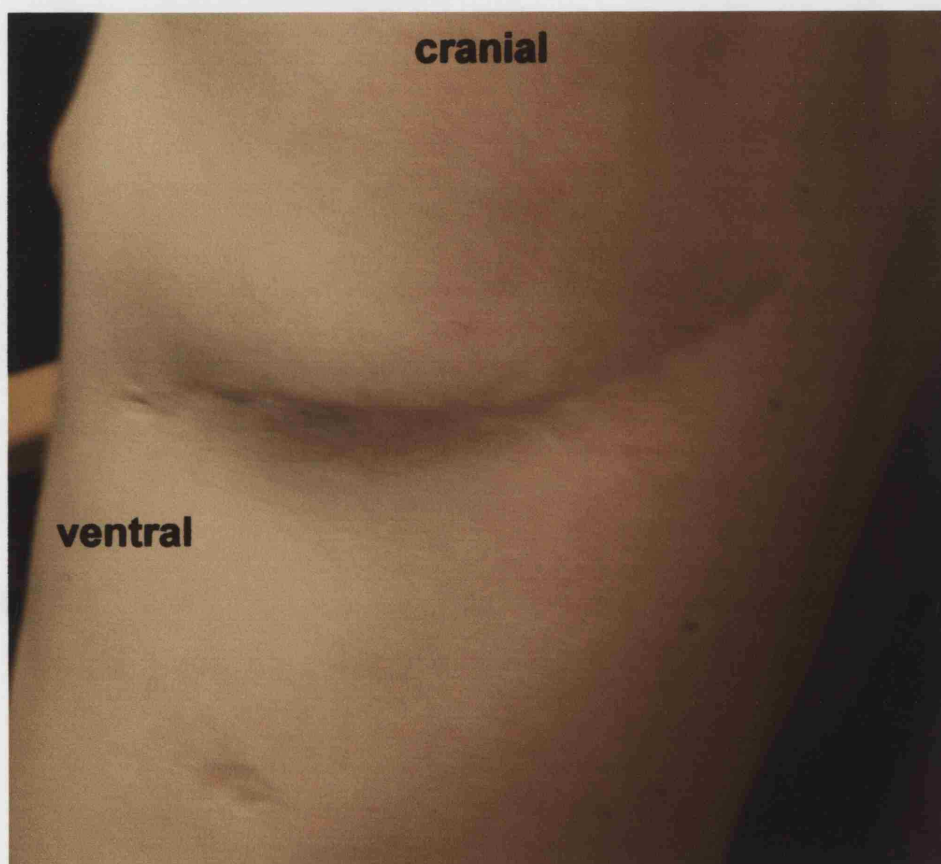


Figure 3.8:
Von Frey perception threshold:
Comparisons scar/thoracic areas

n=9 per group, error bars denote SD

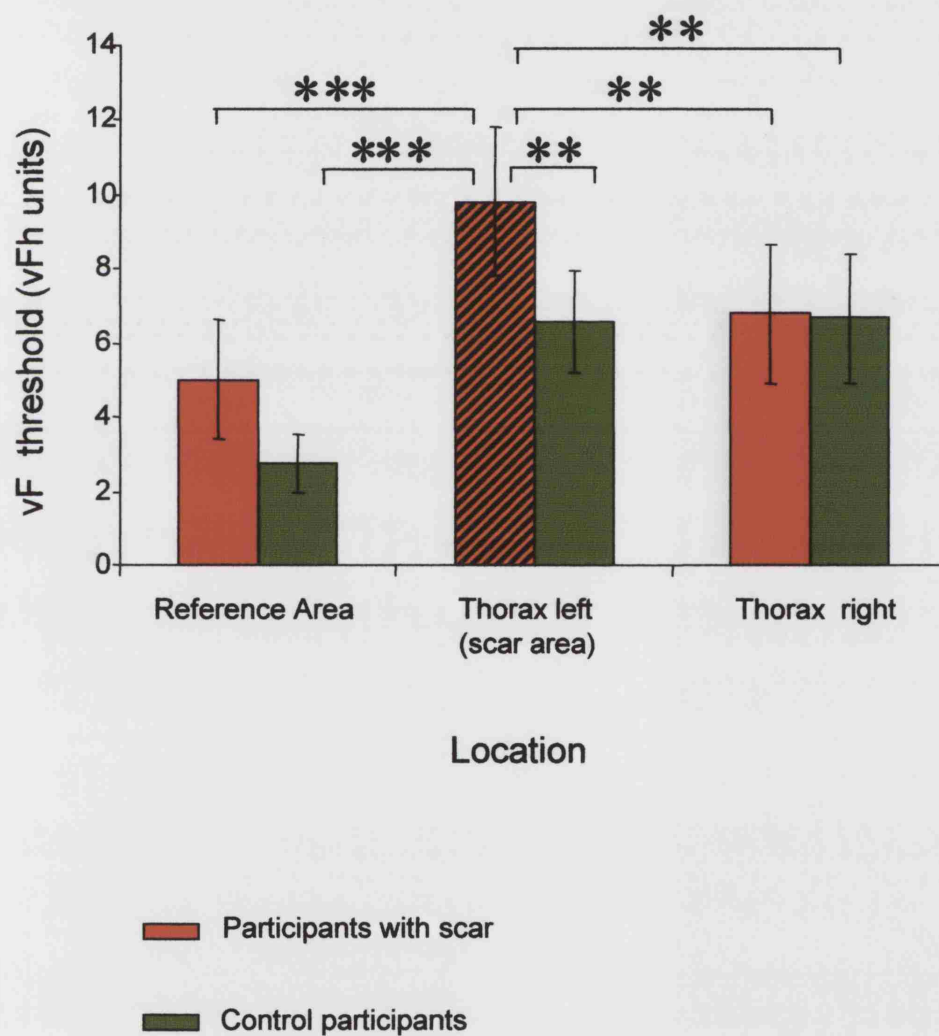


Figure 3.9:
Cool perception:
Comparisons scar/thoracic areas

n=9 per group, error bars denote SD

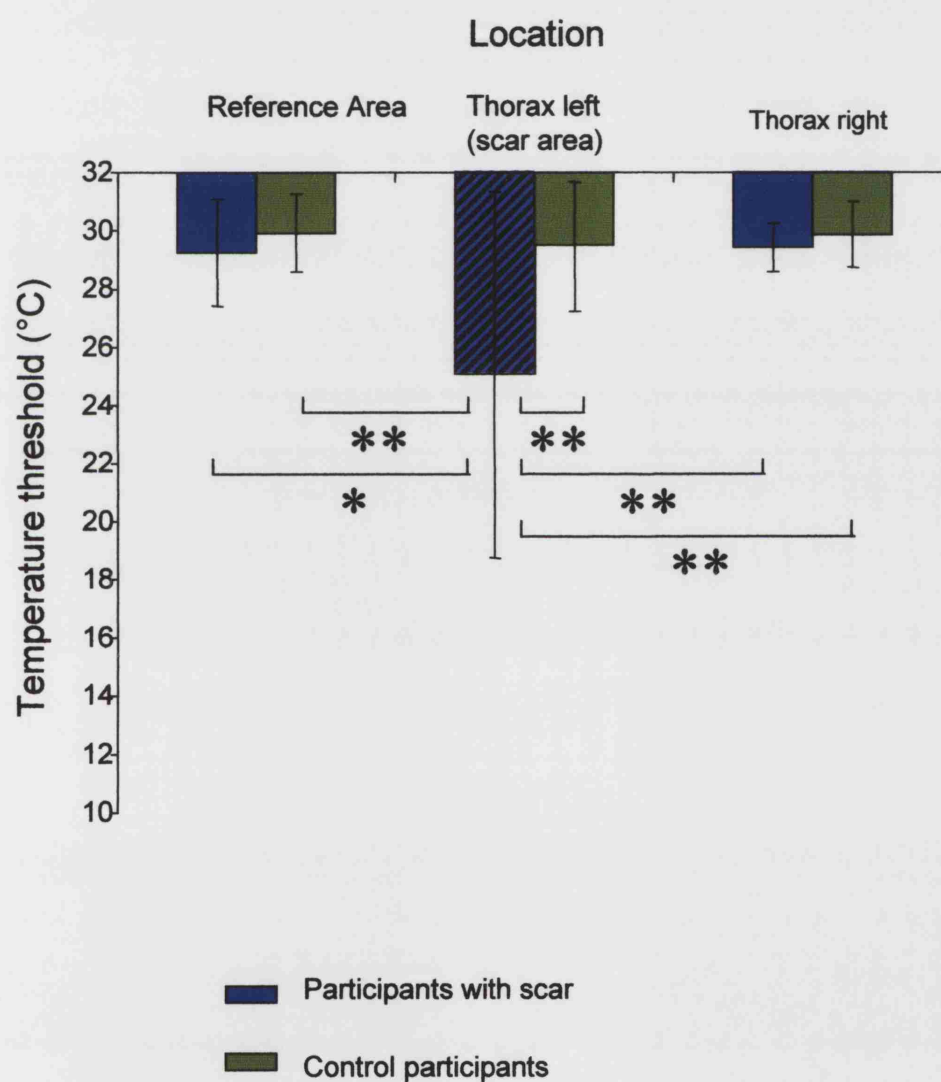


Figure 3.10:
Warm perception:
Comparisons scar/thoracic areas

n=9 per group , error bars denote SD

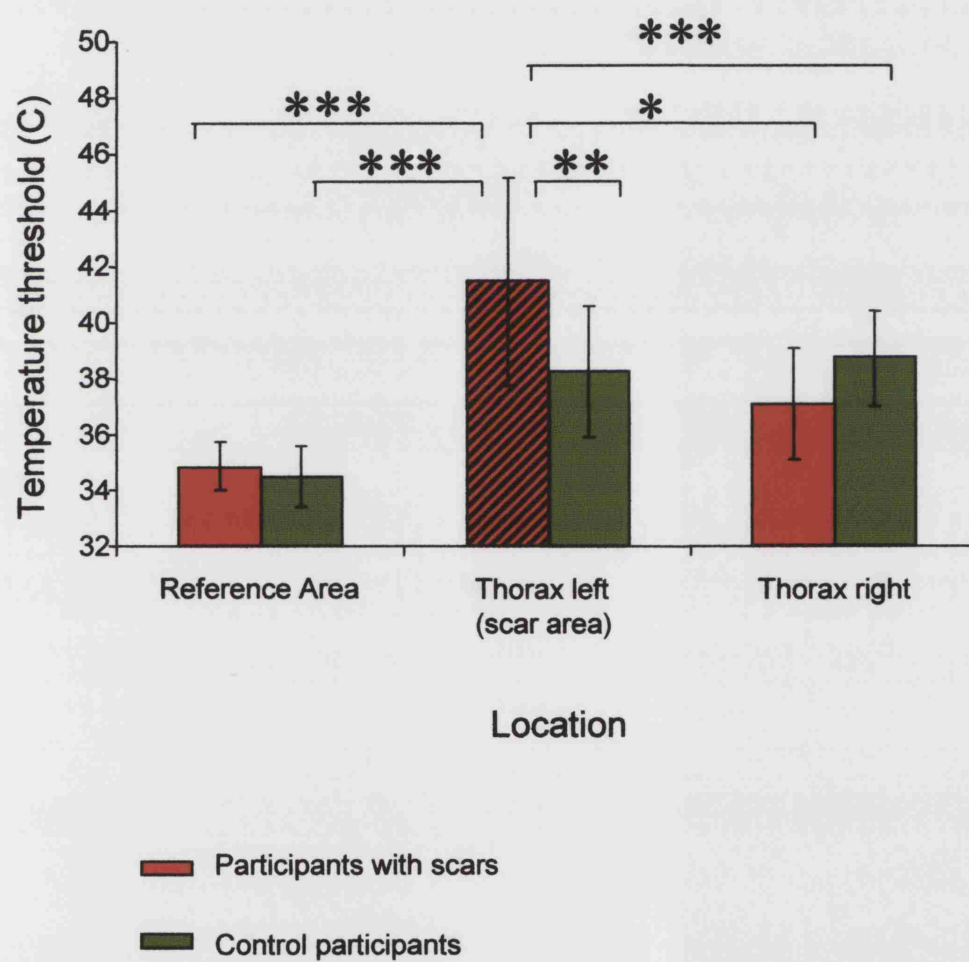


Figure 3.11:
Illustration of warm threshold/sensations

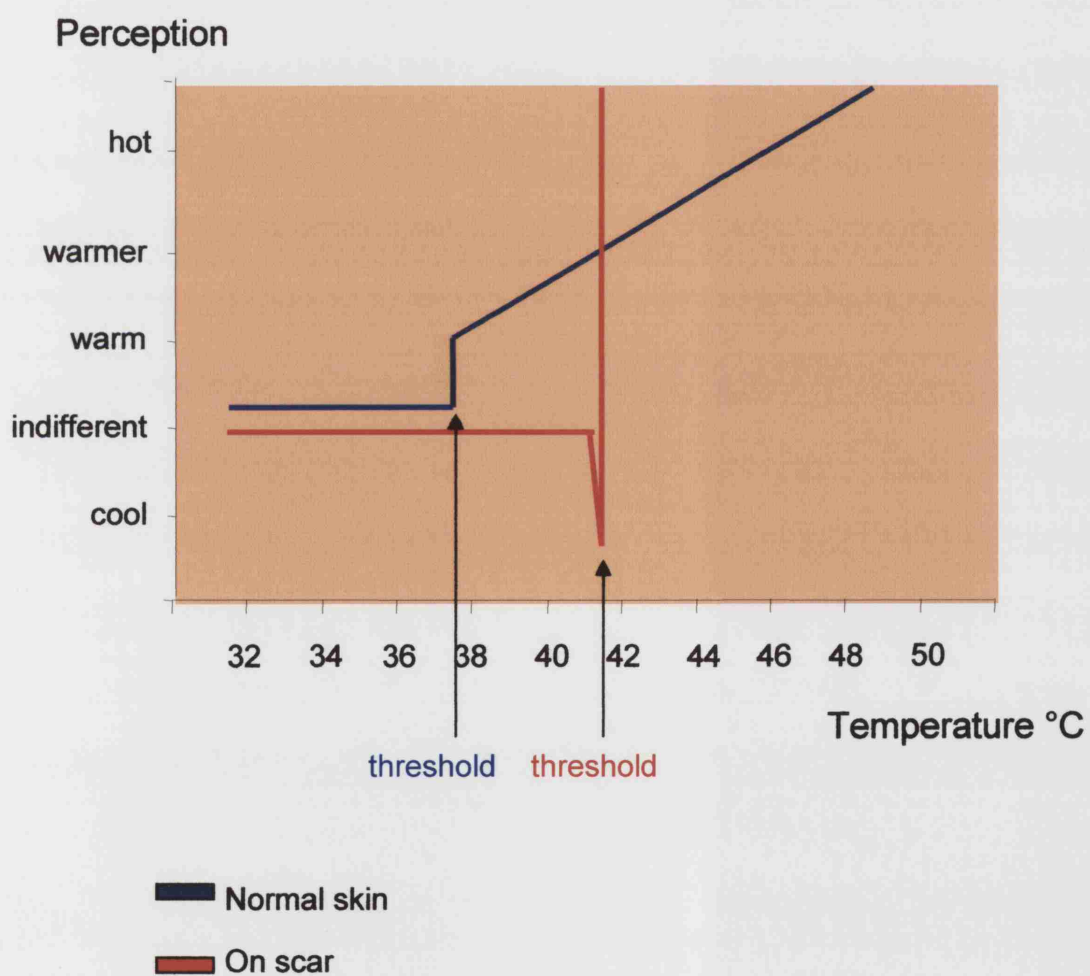


Figure 3.12:
Von Frey perception threshold :
Comparisons reference areas

n=9 per group, error bars denote SD

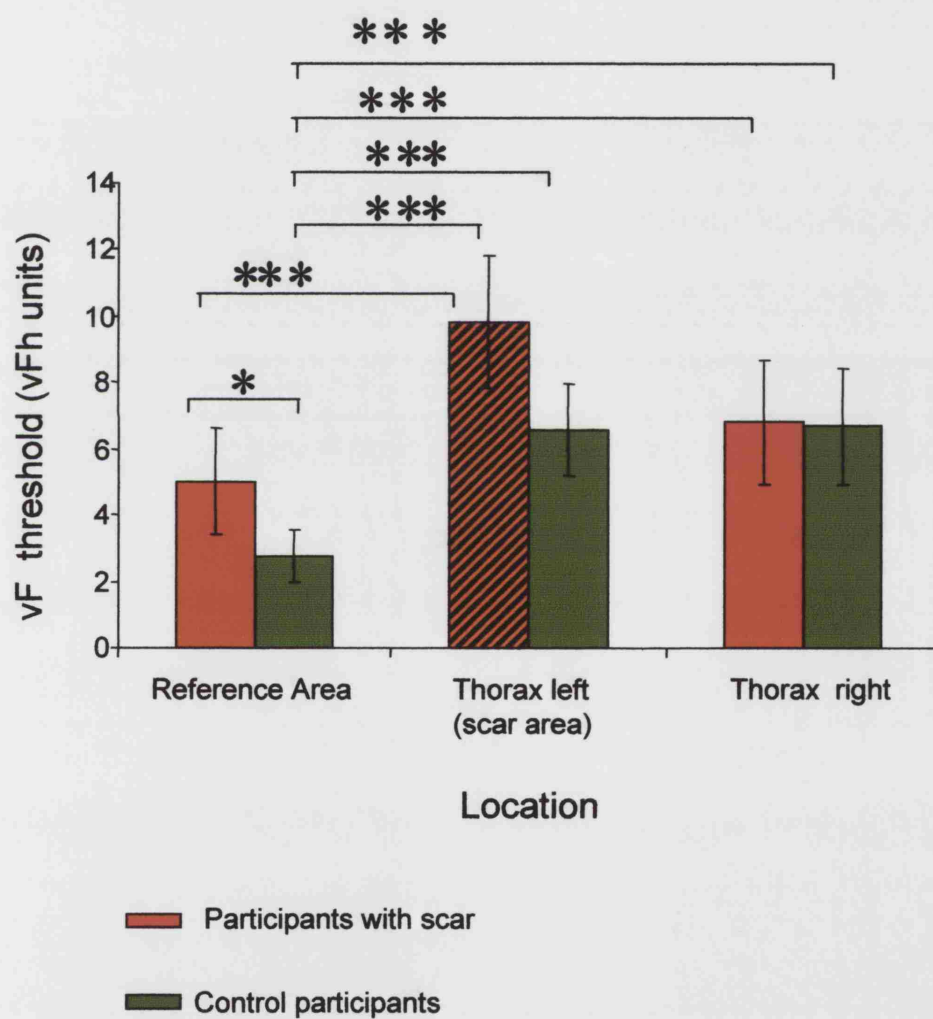


Figure 3.13:
Cool perception:
Comparisons reference areas

n=9 per group, error bars denote SD

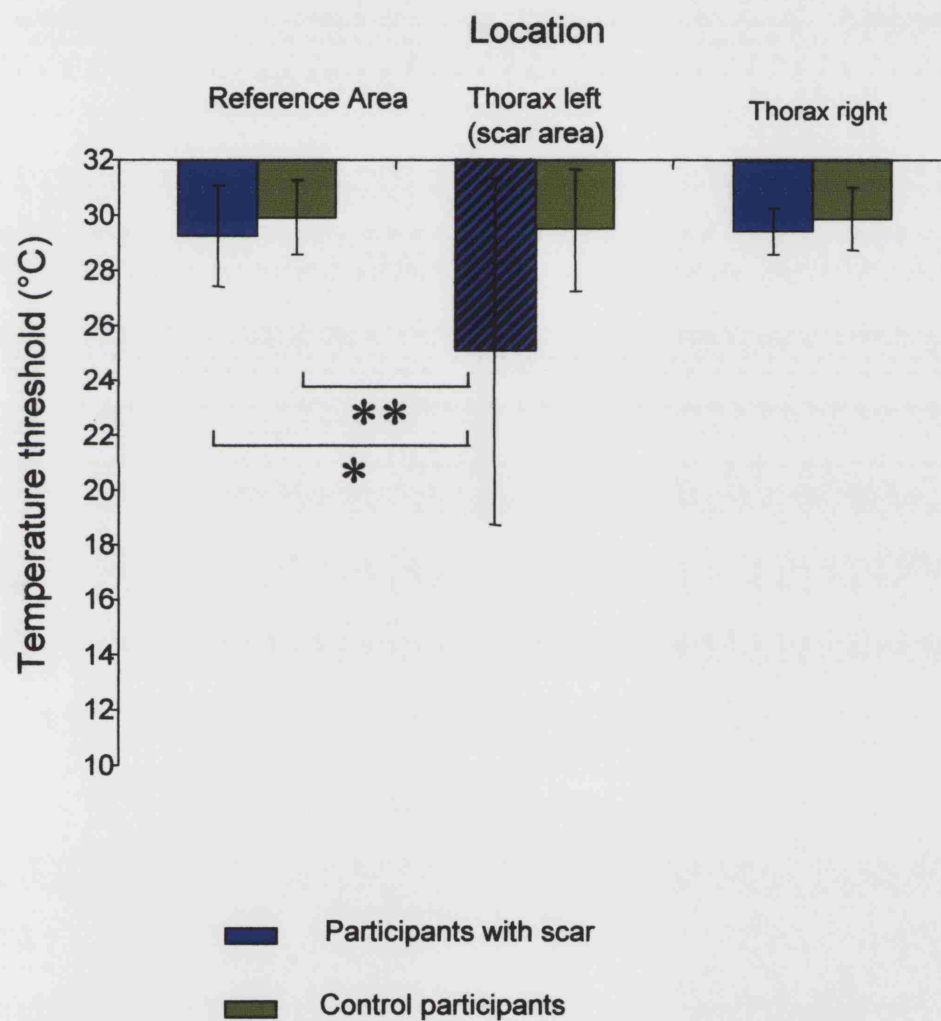
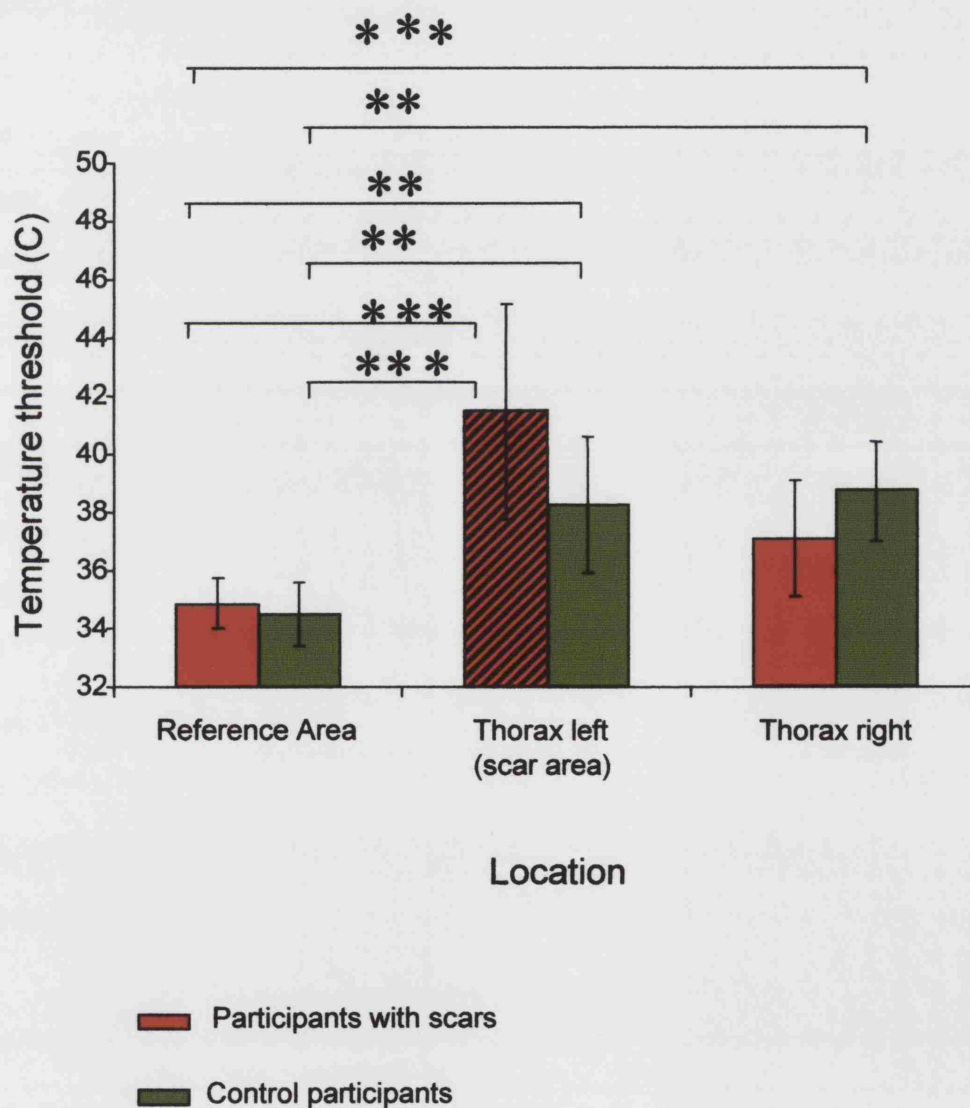


Figure 3.14:
Warm perception:
Comparisons reference areas

n=9 per group , error bars denote SD



4. Conclusions and Directions for the Future

4.1 Conclusion and Significance of findings

We are able to conclude that tissue injured in early infancy remains measurably altered to mechanical and thermal stimulation in later life. QST, as a quantitative and objective method, was used to test the scarred tissue. In the first group we examined, NICU survivors, we found a mixture of alterations, hypo- and hyperesthesia and hypo- and hyperalgesia. In our second group, children with a thoracic scar of neonatal origin, a very clear pattern emerged, which showed decreased sensitivity for touch, cold and warm perception. Furthermore our participants reported that instead of first perceiving “warm”, they instantly felt “hot” over the scar.

Iatrogenic skin damage is an inevitable consequence of neonatal treatment in an intensive care unit and after surgery. In our studies, although we showed objective and statistically significant changes in perception on the damaged skin areas, none of the participants had been aware of such changes or indeed was significantly adversely affected by them. However such changes could potentially adversely affect the individual concerned, depending on location and site of the scarring. Having no gradual appreciation of increasing heat could possibly lead to burning particularly on hands and feet.

Our findings confirm for the first time in humans long-term somatosensory changes over a skin area injured in the neonatal period. There are many animal studies which have addressed and confirmed this issue (e.g. Anand, 1999; Alvares et al., 2000; Lidow et al., 2000; Ren et al., 2004), but human studies to date concentrated mainly

on behavioural responses or deep tenderness (e.g. Taddio et al., 1995,1997; Grunau et al., 1994a,b, 1998; Buskila et al., 2003).

We also found our participants with thoracic scars to be less sensitive to touch on the hand than their age-matched controls. This was not observed in the first study group. This first group however, when questioned, experienced common pains as less painful than their age-matched controls. Consistent with this, some of the children with a thoracic scar told us that they perceive less pain than their friends. These observations confirm the evidence in the literature that early infant injury has not only local, but also global long-term consequences (Ren et al., 2004).

Stress-induced analgesia may be one of the main mechanisms to explain these observations (Fitzgerald, 2004). From our findings one could possibly argue that exposure to a stressful environment in itself, like the adolescents in our NICU survivor group, but without major scarring, may not have been insult enough to result in overall somatosensory changes as well as behavioural changes. Which would mean that for a stressful experience to cause somatosensory changes either the non-somatic insult/stress would have to be more major or it needs to be accompanied by a somatic insult of significant magnitude.

Another point to consider is the age at testing of our two participant groups, the group with baseline somatosensory changes was in average 10 years of age; whereas the second group without was 17 years. It might well be possible that further experiences and learning over time in this group were able to change and readjust the balance of excitation and inhibition in the somatosensory system and therefore readjusted a once existing hypoalgesia (Ren et al., 2004).

We confirmed with our study that QST is a valuable tool for assessment in sensory perception in children and that the method is well tolerated. To achieve optimum results it is important to keep distractions and boredom to a minimum, to be able to keep concentration high and to limit the time of testing according to the age of the examined group.

4.2 Future research

Following on from our study it would be possible to gain valuable information by testing two more groups of children:

- (i) Children of the same age who have received a similar scar later in life e.g. after the age of four. This would allow us to find out if there is any difference in perception of any modality between a neonatal and a later scar.
- (ii) Children who had a neonatal thoracotomy scar, but who are older at the time of testing, this could give two types of information, firstly about changes in the scar sensitivity over time and secondly about changes in the baseline sensitivity over time.

It would be obviously desirable to recruit larger numbers of children for further studies, especially for investigating changes in baseline sensitivity. Keeping in mind all the confounding factors (e.g. age at insult, nature of the injury and possibly the time since injury elapsed at the time of testing) very strict criteria in the setting of a human study will have to be applied to get convincing results. It may be necessary to do multicentre studies for this reason and it might be interesting to group the children according to gestational age at birth, number of invasive procedures and length of intensive care

stay and then follow them over a period of years. Multicentre studies though may be difficult between centres regarding the QST technique, but with strict criteria and the same equipment could possibly be achieved.

Regarding modalities used for testing “hot pain” may be a useful further modality to help investigating fibres involved and mechanisms responsible for changes, also regarding the aberrant “warm sensation” discovered, it would be valuable to distinguish the character, e.g. burning or sharp pricking to gain further information about the mechanisms behind.

Future research to examine consequences of long-term early injury and their mechanisms are all the more important in the light of ever increasing possibilities of medical interventions and the increasing number of babies surviving after having been subjected to them.

Appendix 2.1: GP letter

Confidential

Dr
Forest Road Health Centre
Forest Road
Edmonton
London
N9

6th August 2002

Dear Dr

Re: Contact Tracing Neonatal Follow-Up Programme

M....W.... dob:..... ofwas admitted to the Neonatal Unit at University College London Hospital in 1985 and is a member of a long-term follow-up programme administered by the University College London NHS Trust Hospitals and the Paediatric Pain Research Group.

We would like to contact the children and their parents to ask if they are happy to participate in a research project investigating the long-term consequences of early infant injury and trauma upon somatosensory processing. Clearly, before contacting them we would like to ensure that it is appropriate. If you feel there is any reason why we should not approachand his parents to ask them if they would consent to participating in the project please let us know.

Please could you confirm the family's address and it would be of great help to us if you could also answer the questions about chronic illness/regular medication.

We are most grateful for your help and enclose a prepaid envelope for your reply. If you would like any more information or have any queries please contact me by telephone: 0207 404 9258 or e-mail: b.schmelzle-lubiecki@ich.ucl.ac.uk

Many thanks.

Yours sincerely,

Dr Beate M Schmelzle-Lubiecki MD FRCA
Research Fellow, Paediatric Pain Research Group

Reply Form

Kindly complete this and return in the prepaid envelope

Re: Contact Tracing Neonatal Follow-Up Programme

I confirm that **M.....W.....**'s address and telephone number are **correct¹/ incorrect**

The correct address is:

.....
.....
.....

Postcode.....

Tel.No.:.....

I do know¹/do not know of any reason why you should not approach the above adolescent and her/his parents/guardians to ask them if they would consent to participate in our project.

Comments:.....

.....
.....

M..... W..... as developed¹/has not developed a chronic illness since discharge from the Neonatal Unit.

Details (e.g. Diabetes mellitus age 5):

.....
.....

M..... W..... is taking¹/is not taking regular medication.

Details:.....

.....
.....

Name:

Signature:

¹ Delete as applicable

Appendix 2.2: Invitation letter NICU survivors

Name
Address

16.07.2002

Dear.....,

We are writing to you because you have been admitted to the Neonatal Unit at University College London Hospital in 1985 as a baby and you are a member of a long-term follow-up programme administered by the University College London NHS Trust Hospitals and the Paediatric Pain Research Group.

We are currently involved in a research project looking at the long-term effects of injury as a young baby on sensation in later life, and are asking you whether you would be prepared to be involved in our study.

The aims of the project are, firstly, to see whether people who have had an operation and/or intensive care as babies have changed sensation, especially in areas of skin which were injured as a result of the operation/intensive care. Secondly, we wish to see whether the same people will say that they have unusual pain experience and reactions in and around the affected skin. This work will extend our knowledge and improve the quality of life of sick newborn babies receiving medical treatment, and will help us to know how to prevent long-term damage to sensation in these babies. Over time, our research may lead to improved developmental and health outcomes, and reduce disability in children.

Should you decide to participate in the study, you will be invited to come to the University College Hospital for about 1 hour of testing of your sensation of touch and temperature. The testing is not unpleasant and is most definitely not harmful, but obviously we will stop any time if you do not feel comfortable or happy. We will also ask you some questions about experience with pain and about general health. We would pay you the sum of £20 for participating in the study, and also pay your expenses for the trip to the hospital.

If you are interested in participating in the study, please reply using the enclosed reply sheet and stamped addressed envelope. Once I receive your reply sheet I will send you a more detailed information sheet and we can arrange a time for the testing.

Many thanks for reading this letter, and we look forward to hearing from you.

With best wishes

Yours sincerely,

Dr Beate Schmelzle-Lubiecki, Research fellow.

I, S.....P....., am interested in participating in the study about the long-term effects of injury as a baby on sensation in later life.

I prefer to be contacted by

- a. normal mail
- b. e-mail, my address is:
- c. telephone, my number is:

Please circle what is best for you and we will contact you after sending you more detailed information about the study.

Signature:

Appendix 2.3: Second contact letter with information leaflet NICU survivors

Name
Address

29.08.2002

Dear P...,

Thank you very much for your reply concerning our study about the long-term effects of injury as a baby on sensation in later life.

Please find enclosed some more detailed information. Do not hesitate to contact me if you have any further questions.

To arrange a convenient date and time for the testing please call me on 07771 768332 (any time Monday to Friday between 8 am and 9 pm), I will then call you back.

I look forward to hearing from you soon.

With best wishes

Yours sincerely,

Dr Beate Schmelzle-Lubiecki

PATIENT INFORMATION

TITLE OF PROJECT

Long term consequences of early infant injury and trauma upon somatosensory processing (the way in which you feel things like touch, heat and cold on your skin).

THE AIMS OF THE STUDY

(i) To see whether people who have received intensive care as babies feel touch, temperature and pain either more or less than people of the same age who have not had in intensive care, especially in areas of skin which were injured because of intensive care.

(ii) To see whether the same people will report unusual pain experiences and sensations in and around the affected skin.

WHY IS THE STUDY BEING DONE?

Many sick babies and young children experience pain in hospital as a result of illness or treatment.

Recent research has shown that if you have a lot of pain as a baby, this may change the way that the nervous system develops. While every effort is made to give babies needing intensive care adequate pain relief at the time, we do not know if this early pain affects the way you feel touch and pain later on in life.

Therefore, we are testing young people who had intensive care as babies to find out whether they feel touch and pain differently in areas of skin that were damaged as a baby. As a result of this study, we will be in a better position to design ways of preventing such long-term changes in sensation in future. Meanwhile, we will share the results of our research immediately with doctors and nurses to improve the treatment and prevention of pain in these babies.

HOW IS THE STUDY BEING DONE?

If you decide to participate in this study, firstly, as part of a questionnaire, we would like to find out whether you have any areas of scarring as a result of the intensive care that you received as a baby. Then we would like to test your sensation of touch, pinprick and temperature, in and around a scar, and on the other, unaffected side of your body, and finally we will do the same testing in another, unaffected skin area such as the inside of your forearm.

The threshold for both light touch and a pricking sensation will be tested with small probes called von Frey hairs that touch the surface of the skin. By touching the skin for 1 second with each of these hairs, we will measure how quickly you feel each of the two different sensations. This will be repeated 5 times. To test your feeling for temperature a pad giving five warm, five cool, four hot and four cold sensations will be gently placed on your skin. Testing for all sensations will take between 1 and 2 hours.

Please tell us if you have taken pain-relieving tablets within 48 hours of your testing appointment. We request that you do not take alcohol or other substances for 12hrs or smoke for 2 hours before the testing, as all of these things will affect the results of the testing.

ARE THERE RISKS AND DISCOMFORTS?

All the equipment is made to the highest safety standards, and none of the tests cause any damage at all, so there are no risks to the testing. We will be measuring pricking, hot and cold sensations, but as the tests stop as soon as you feel any pain, any discomfort caused by the testing will be minimal. We have already tested people of your age, and they did not have any problems at all with the testing.

discomfort caused by the testing will be minimal. We have already tested people of your age, and they did not have any problems at all with the testing.

WHAT ARE THE POTENTIAL BENEFITS?

This work will give us a greater knowledge on how we can improve the quality of life of sick babies receiving medical treatment, and will help us to prevent long-term problems in sensation in these babies. Over time, our research may lead to improved developmental and health outcomes in children.

WILL THERE BE ANY REWARD FOR PARTICIPATION IN THE STUDY?

The sum of £20 plus expenses will be paid to all those taking part in the study.

WHO WILL HAVE ACCESS TO THE CASE/RESEARCH RECORDS?

All proposals for research using people are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the Joint UCL/UCLH Committees on the Ethics of Human Research. The researchers and a representative of the Ethics Committee will have access to the research records.

You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason. Your decision whether to take part or not will not affect your care and management in any way.

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or if you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee by post via the Ethics Committee Administrator, Research & Development Office, UCLH NHS Trust, 1st floor, Vezey Strong Wing, 112 Hampstead Road, London NW1 2LT, and the administrative staff will put you in contact with him.

HOW TO CONTACT YOUR RESEARCHER:

The name of the researcher is Dr Beate Schmelzle-Lubiecki. You can contact her by
telephone: e-mail: or
write to her at:

Neonatal Unit
Elizabeth Garrett Anderson Hospital
Huntley Street
London
WC1E 6DH

Thank you for considering taking part in our research project.

Appendix 2.4: Pain Questionnaire

QST No.:

Date:

Pain Questionnaire

A. Personal Data:

Name:

Date of birth:

Gender:

If applicable:

Date of last menstrual period:

Periods regular
 irregular

Height:

Weight:

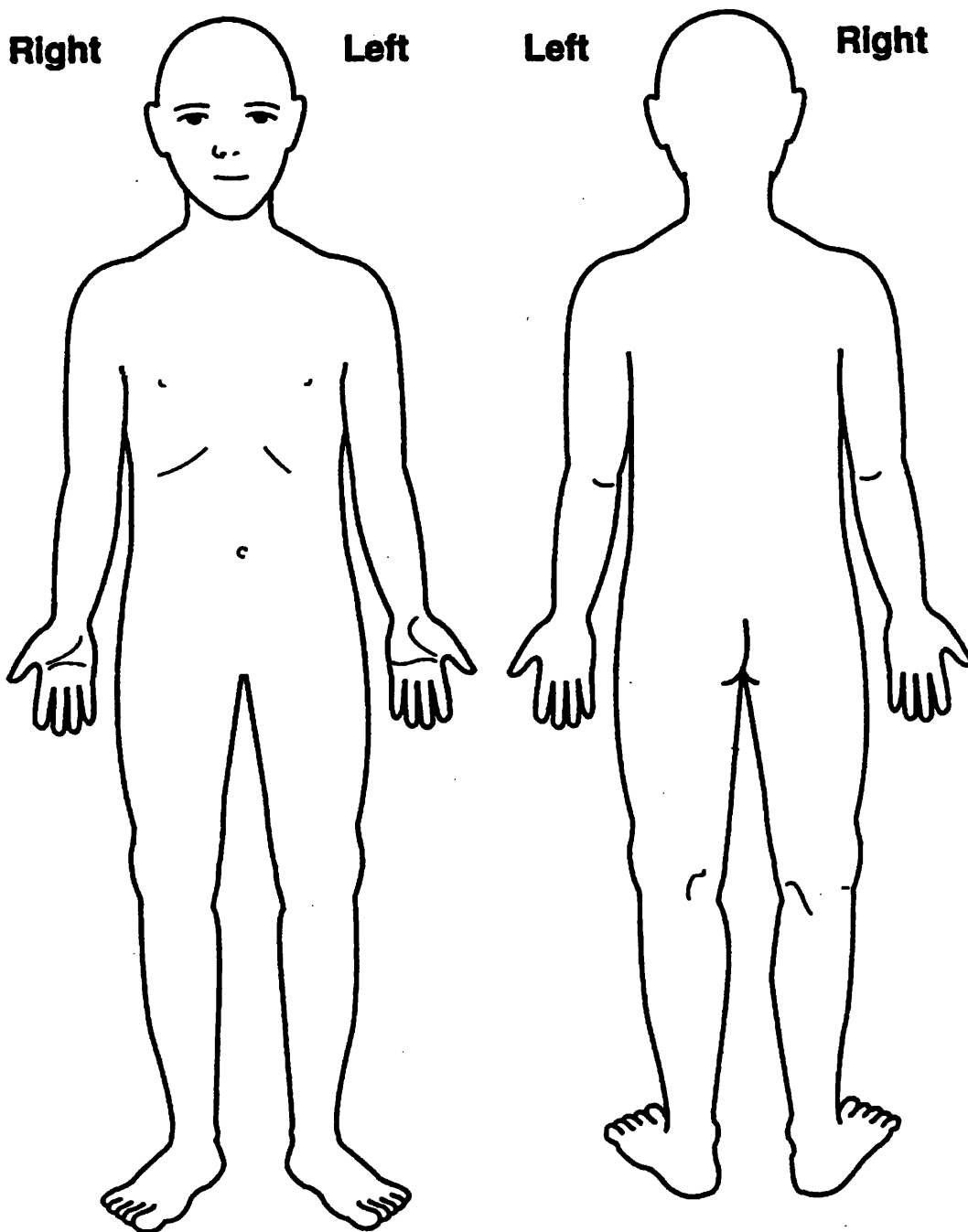
Please tick:

Right handed
Left handed

QST No.:

B. Scarring

Do you have any scars? If yes, please mark on the diagram below where they are and number them.



QST No.:

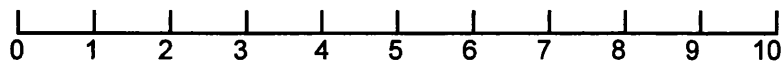
Scar No.:

1. What caused this scar?

2. How old were you when it happened?

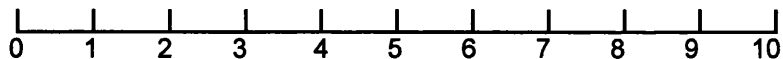
3. Apart from when it happened has it ever been painful? Yes/No

If yes, mark on this scale the amount of pain (0 = no pain/ 10 = worst pain imaginable):



4. Is it painful now? Yes/No

If yes, how painful (0 = no pain/ 10 = worst pain imaginable):



5. Did you ever have any funny feelings/sensations around or on the scar?

Yes/No

**If yes, can you describe these feelings using any of the words below?
Please delete as appropriate:**

Numbness Yes/No

Skin feels thick Yes/No

Pins and needles Yes/No

Itching Yes/No

QST No.:

Tingling Yes/No

Prickly heat Yes/No

Other, please describe:

6. Is the scar ever

Swollen Yes/No

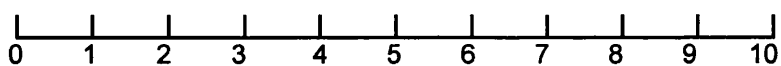
Red Yes/No

7. Is there anything that makes the scar/ area around the scar uncomfortable

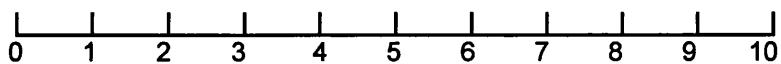
Yes/No

Here is a list of possible triggers, please tick which apply and then rate how much they bother you (0 = not at all/ 10 = worst possible):

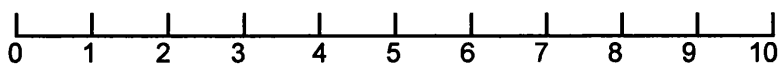
Clothes



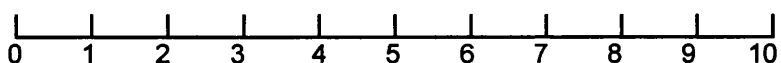
Socks



Bra

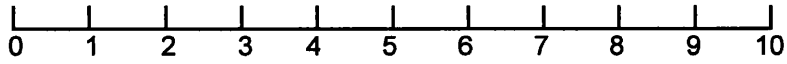


Bedclothes

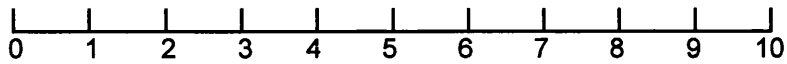


QST No.:

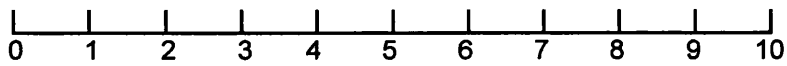
Wool



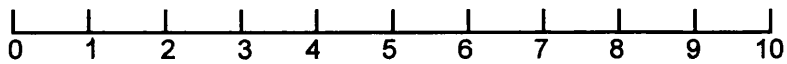
Cold e.g. ice



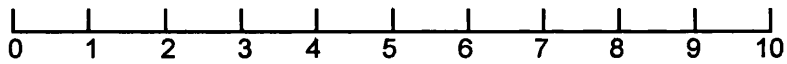
Hot Shower



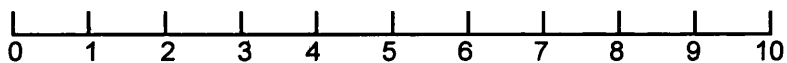
Heat e.g. sitting close to a fire



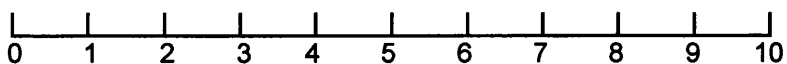
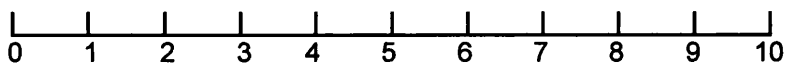
Stress



Tiredness



Other, please specify:



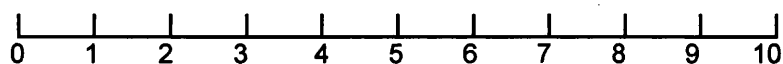
QST No.:

8. Is there anything that makes the scar/ area around the scar painful?

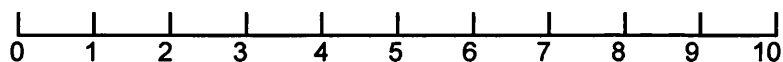
Yes/No

Here is a list of possible triggers, please tick which apply and then rate how much pain they can cause you (0 = no pain/ 10 = worst pain imaginable):

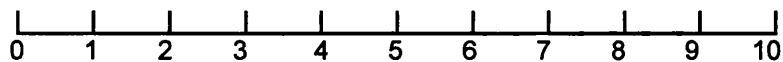
Clothes



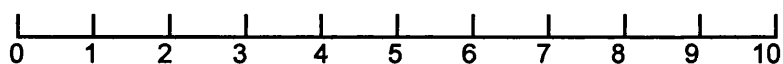
Socks



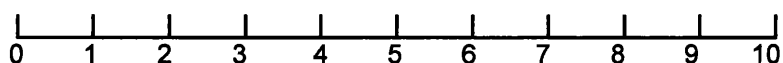
Bra



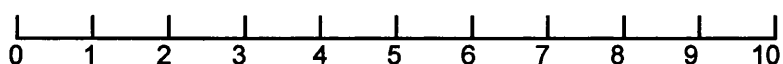
Bedclothes



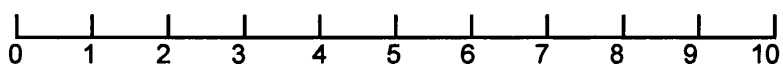
Wool



Cold e.g. ice

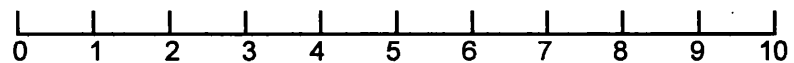


Hot Shower

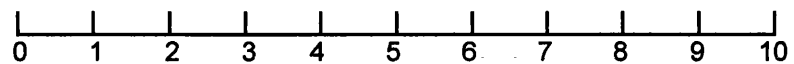


QST No.:

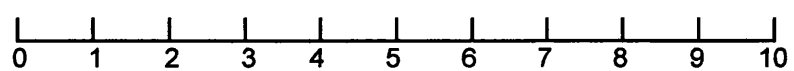
Heat e.g. sitting close to a fire



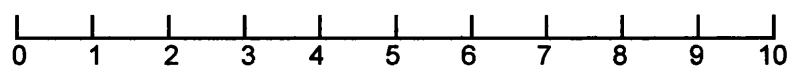
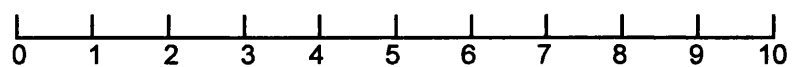
Stress



Tiredness



Other, please specify:

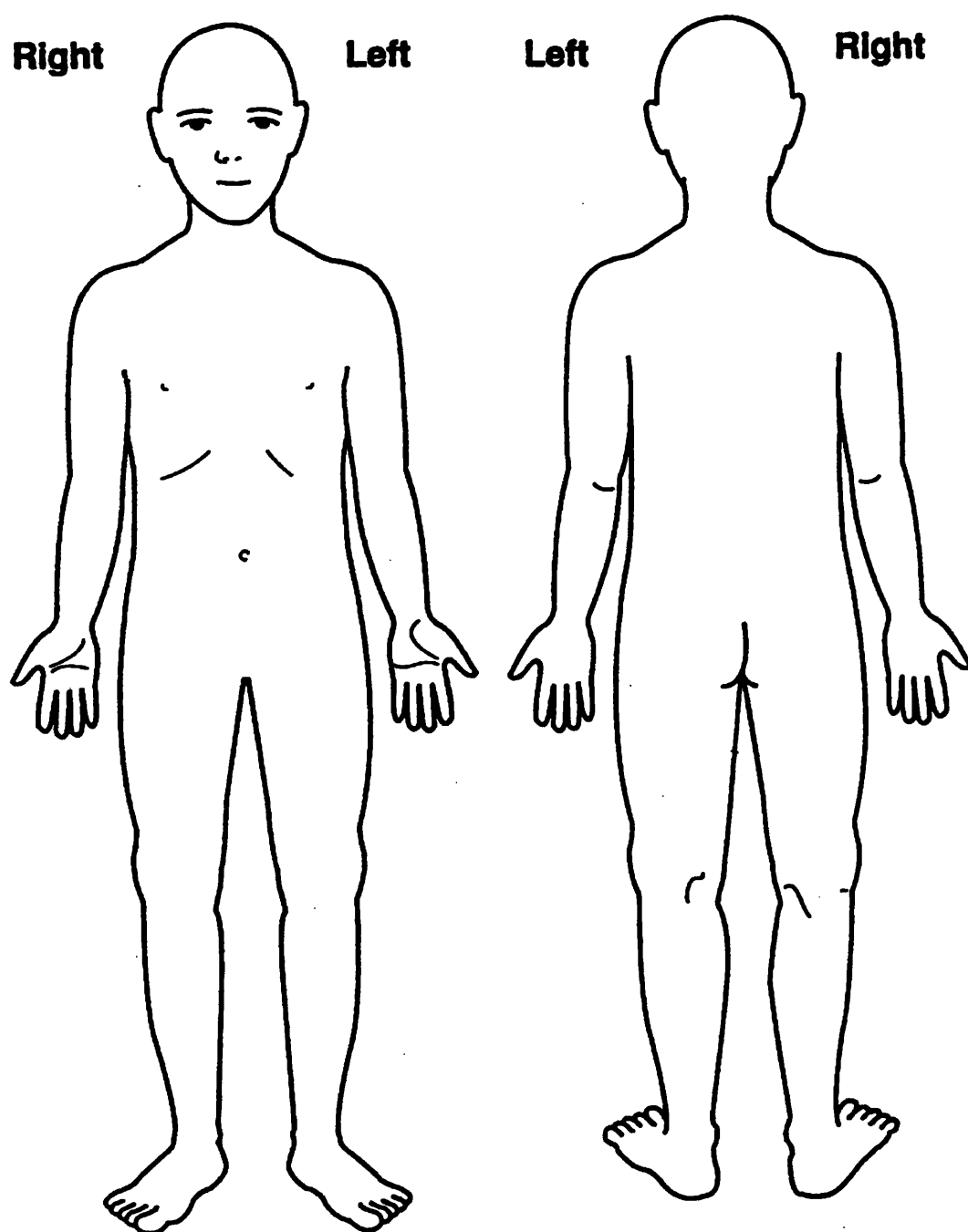


QST No.:

Are there any areas of your skin where you think the sensation is different to all the other skin of the body when you touch it either all the time or part of the time?

Yes/No

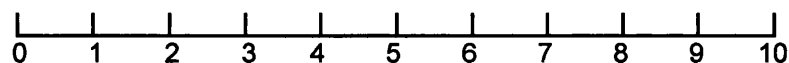
If yes, please mark them on the diagram below:



C. General Pain

1. Do you think you experience more pain than your friends?
Yes/No

If yes, how worrying is the pain for you? Please rate and mark on the line
(0 = not at all/ 10 = most worrying)

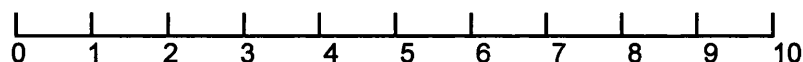


2. Do you have pain that interferes with the things you would like to do in life?

Yes/No

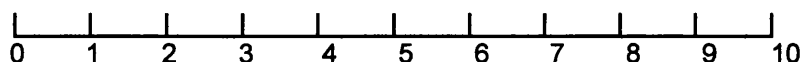
If yes, please explain:

How much does it interfere? Please rate and mark on the line (0 = not at all/ 10 = worst possible)



3. What was the pain you most remember in your life?

If you remember, how painful was it for you? Please rate and mark on the line (0 = no pain/10 = worst pain imaginable):



QST No.:

4. Have you ever suffered from any of the conditions listed below?

If yes, please

- a. circle the appropriate letter to indicate how often you have this type of pain (closest estimate possible):

D = daily

W = weekly

M = monthly

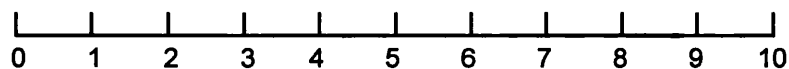
L = less frequently than monthly

- b. rate on the scales below how bad it was at the time of your worst experience with this pain (0 = no pain/ 10 = worst pain imaginable) and state your age when it happened:

Headache

yes/no

D W M L

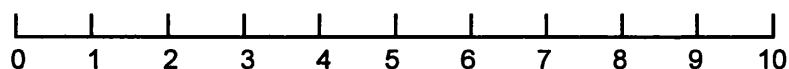


age:

Toothache

yes/no

D W M L

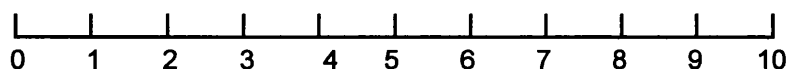


age:

Earache

yes/no

D W M L



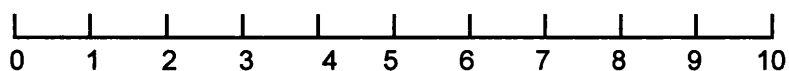
age:

Sprains,

e.g. due to sports injury

yes/no

D W M L



age:

QST No.:

D = daily

W = weekly

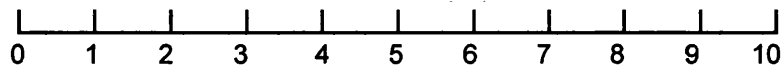
M = monthly

L = less frequently than monthly

Bruises

yes/no

D W M L

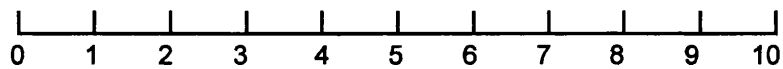


age:

Backache

yes/no

D W M L

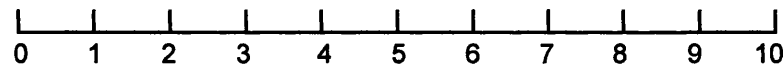


age:

Joint Pain

yes/no

D W M L



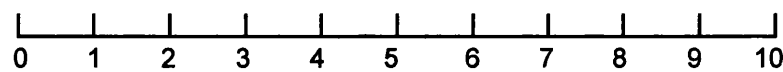
age:

Abdominal Pain

yes/no

D W M L

(Tummy ache)

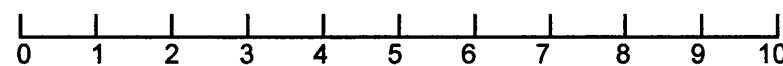


age:

Period Pain

yes/no

D W M L



age:

QST No.:

C. General Health

1. Have you ever had any injuries that needed treatment in hospital?

Yes/No

when

what

2. Have you ever had any operations?

Yes/No

when

why

3. Medication

Regular medication at present

Yes/No

Regular medication in the past

Yes/No

Intermittent

Yes/No

Over-the-counter medication

Yes/No

QST No.:

4. Are you allergic to anything? Yes/No

5. Do you have or have you ever had any of the following?

Breathing problems? Yes/No

Heart problems? Yes/No

Kidney problems? Yes/No

Blood disorders? Yes/No

Convulsions or fits? Yes/No

QST No.:

D. Social Background

Year in school/place of work:

When did your mother leave full-time education? Please add her age if you remember:

After: School
 College
 University

Mother's profession:

When did your father leave full-time education? Please add his age if you remember:

After: School
 College
 University

Father's profession:

Ethnic origin, please tick all which are appropriate:

White	Indian
Black Caribbean	Pakistani
Black African	Bangladeshi
Black Other	Chinese

Any other ethnic group, please name:

QST No.:

PRIVATE and CONFIDENTIAL

This information will not be disclosed to anybody!

Please be honest as **we will not tell anyone else about this**, but the substances mentioned below influence our test results!

Have you been using any of the substances below for recreation or as a habit?
Please tick where appropriate:

	within the last month	within the last
12 hours		
Cigarettes	()	()
Alcohol	()	()
Cannabis	()	()
Ecstasy	()	()
Amphetamines i.e. speed	()	()
LSD/magic mushrooms	()	()
Cocaine	()	()
Crack	()	()
Heroin	()	()
Valium or other downer	()	()
Solvents i.e. gas, glue, lighter fluid	()	()

Any other drugs not mentioned above, please specify:

Appendix 3.1: GP letter Cardiac Surgery Group

Confidential

Dr
Forest Road Health Centre
Forest Road
Edmonton
London
N9

25th June

2003

Dear Dr

Re: Contact tracing for research project

J.....B..... dob: of, was admitted to the Cardiothoracic Unit at Great Ormond Street Hospital in 1991 for surgery.

We would like to contact the children and their parents to ask if they are happy to participate in a research project investigating the long-term consequences of early infant injury and trauma upon somatosensory processing. Clearly, before contacting them we would like to ensure that it is appropriate. If you feel there is any reason why we should not approach and his/her parents to ask them if they would consent to participating in the project please let us know.

Please could you confirm the family's address and it would be of great help to us if you could also answer the questions about chronic illness/regular medication.

We are most grateful for your help and enclose a prepaid envelope for your reply. If you would like any more information or have any queries please contact me by telephone: or e-mail:

Many thanks.

Yours sincerely,

Dr Beate M Schmelzle-Lubiecki MD FRCA
Research Fellow, Paediatric Pain Research Group

Reply Form

Kindly complete this and return in the prepaid envelope

Re: Contact tracing for somatosensory testing

I confirm that¹ s address and telephone number are **correct¹/ incorrect**

The correct address is:

.....

.....Postcode.....

Tel.No.:.....

I do know¹/do not know of any reason why you should not approach the above child and her/his parents/guardians to ask them if they would consent to participate in our project.

Comments:.....

.....

.....

..... has developed¹/has not developed a chronic illness since discharge from the Cardiothoracic Unit.

Details (e.g. Diabetes mellitus age 5):

.....

.....

.....

..... is taking¹/is not taking regular medication.

Details:.....

.....

.....

Name:

Signature:

¹ Delete as applicable

Appendix 3.2:

Invitation Letter and Information Leaflet Cardiac Surgery Group

Parent/Guardian of
Addresss

16.07.2003

Dear Parent/Guardian of,

We are writing to you because you child had an operation as a newborn baby at Great Ormond Street Hospital. We are currently involved in a research project looking at the long-term effects of injury as a young baby on sensation in later life, and are asking you whether you and your child would be prepared to be involved in our study.

Should you decide to participate in the study, you and your child will be invited to come to the University College Hospital for about 1 hour of testing. The testing is not unpleasant and is most definitely not harmful, but obviously we will stop any time if your child does not feel comfortable or happy. Please find enclosed a detailed information sheet about the study. We would pay the sum of £20 for participating in the study, and also pay your expenses for the trip to the hospital.

If you and your child are interested in participating in the study, please reply using the enclosed reply sheet and stamped addressed envelope. We will be doing testing every day from 30.07.03 to 10.08.03. If this is not convenient for you we can also arrange a later date. Once we receive your reply sheet we will contact you answering any questions you might have and confirming the appointment time.

Many thanks for reading this letter, and we look forward to hearing from you.

With best wishes

Yours sincerely,

Dr Beate Schmelzle-Lubiecki, Research fellow.

I,, and my child are interested in participating in the study about the long-term effects of injury as a baby on sensation in later life.

Contact telephone number:

We would be able to come for testing at the following days (please tick):

Wednesday, 30.07.:	am	pm
Thursday, 31.07.:	am	pm
Friday, 01.08.:	am	pm
Saturday, 02.08.:	am	pm
Sunday, 03.08.:	am	pm
Monday, 04.08.:	am	pm
Tuesday, 05.08.:	am	pm
Wednesday, 06.08.:	am	pm
Thursday, 07.08.:	am	pm
Friday, 08.08.:	am	pm
Saturday, 09.08.:	am	pm
Sunday, 10.08.:	am	pm

None of these times is convenient, but we would be able to attend at a later date.

Signature:

PARENT/GUARDIAN INFORMATION

TITLE OF PROJECT

Long term consequences of early infant injury and trauma upon somatosensory processing (the way in which one feels things like touch, warm and cool on the skin).

THE AIMS OF THE STUDY

(i) To see whether people who have received intensive care or had operations as babies feel touch, temperature and pain either more or less than people of the same age who have not had in intensive care, especially in areas of skin which were injured because of intensive care.

(ii) To see whether the same people will report unusual pain experiences and sensations in and around the affected skin.

WHY IS THE STUDY BEING DONE?

Many sick babies and young children experience pain in hospital as a result of illness or treatment.

Recent research has shown that if you have a lot of pain as a baby, this may change the way that the nervous system develops. While every effort is made to give babies needing operations and intensive care adequate pain relief at the time, we do not know if this early pain affects the way you feel touch and pain later on in life.

Therefore, we are testing children who had an operation and/or intensive care as babies to find out whether they feel touch and pain differently in areas of skin that were damaged as a baby. As a result of this study, we will be in a better position to design ways of preventing such long-term changes in sensation in future. Meanwhile, we will share the results of our research immediately with doctors and nurses to improve the treatment and prevention of pain in these babies.

HOW IS THE STUDY BEING DONE?

If you decide to participate in this study we would like to test your child's sensation of touch and temperature, in and around the scar on your child's chest which it has from his/her operation when he/she was a baby, and on the other, unaffected side of the body, and finally we will do the same testing in another, unaffected skin area such as the inside of the forearm.

The threshold for touch will be tested with special plastic hairs called von Frey hairs that touch the surface of the skin. By touching the skin for 1 second with each of these hairs, we will measure how quickly this sensation is felt. This will be repeated 5 times. Another test for touch will be brushing of the skin with a thick, soft paint brush. To test the feeling for temperature a pad giving five warm and five cool sensations will be gently placed on the skin. Testing for all sensations will take about 1 hour and takes place in the Neonatal Unit at the University College London Hospital.

Please tell us if your child has taken pain-relieving tablets within 48 hours of the testing appointment.

ARE THERE RISKS AND DISCOMFORTS?

All the equipment is made to the highest safety standards, and none of the tests cause any damage at all, so there are no risks to the testing. We have already tested people of your age, and they did not have any problems at all with the testing.

This work will give us a greater knowledge on how we can improve the quality of life of sick babies receiving medical treatment, and will help us to prevent long-term problems in sensation in these babies. Over time, our research may lead to improved developmental and health outcomes in children.

The sum of £20 plus expenses will be paid to all those taking part in the study.

All proposals for research using people are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the Joint UCL/UCLH Committees on the Ethics of Human Research. The researchers and a representative of the Ethics Committee will have access to the research records.

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or if you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee by post via the Ethics Committee Administrator, Research & Development Office, UCLH NHS Trust, 1st floor, Vezey Strong Wing, 112 Hampstead Road, London NW1 2LT, and the administrative staff will put you in contact with him.

The name of the researcher is Dr Beate Schmelzle-Lubiecki. You can contact her by telephone: e-mail: or write to her at:

Thank you for considering taking part in our research project.

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